ASSOCIATION OF CHOLESTERYL ESTER TRANSFER PROTEIN GENE TaqIB POLYMORPHISM AND THE ASSOCIATED PHENOTYPE VARIATION WITH CORONARY ARTERY DISEASE

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **ASSOCIATION OF CHOLESTERYL ESTER TRANSFER PROTEIN GENE TaqIB POLYMORPHISM AND THE ASSOCIATED PHENOTYPE VARIATION WITH CORONARY ARTERY DISEASE** is the original bona fide work done by **Dr.V.Umamaheswari,** Post Graduate Student Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

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ABBREVIATION

CHD	–	Coronary Heart Disease
VLDL	–	Very Low Density Lipoprotein
LDL	–	Low Density Lipoprotein
HDL	–	High Density Lipoprotein
CETP	–	Cholesteryl ester transfer protein
Mr	–	Relative molecular mass
ICAM	–	Intercellular Cell Adhesion Molecule 1
VCAM1	–	Vascular Cell Adhesion Molecule 1
PeCAM1	–	Pericellular Cell Adhesion Molecule1
IL1	–	Interleukin 1
TNF-α	–	Tumour Necrosis Factor-α
PDGF	–	Platelet Derived Growth Factor
FGF	–	Fibroblast Growth Factor
ROS	–	Reactive Oxygen Species
eNOS	–	Endothelial Nitric Oxide Synthase
LBP	–	Lipopolysaccharide-binding protein
BPI	–	Bactericidal permeability-increasing protein

mRNA	–	Messenger RNA
LVH	–	Left venticular hypertrophy
DM	–	Diabetes mellitus
HYT	–	Hypertension
SMK	–	Smoking
ALC	–	Alcoholism
WT	–	Weight
HT	–	Height
BMI	–	Body Mass Index
CHOL	–	Cholesterol
TGL	–	Triglyceride
EDTA	–	Ethylene Diamine Tetra Acetic Acid
DNA	–	Deoxyribonucleic acid
cDNA	–	Complementary DNA

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INTRODUCTION

INTRODUCTION

Coronary artery disease (CAD) has become a major public health problem in many developing countries[1,2]. CAD is a multifactorial disease caused by genetic and environmental factors [3]. Lipoproteins play a central role in the development of atherosclerotic cardiovascular disease in humans. The protective role of high density lipoprotein cholesterol (HDL-C) against atherosclerosis is well established[6,7]. Numerous genetic, hormonal and environmental factors determine HDL-C levels within distinct populations. HDL exerts its cardioprotective function through a process called reverse cholesterol transport, in addition to anti-inflammatory and antioxidative effects. Reverse cholesterol transport describes a metabolic pathway initiated by HDL-mediated efflux from peripheral tissues and subsequent delivery to the liver[21,22]. The cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL metabolism and in reverse cholesterol transport [23,24.]

Cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein composed of 476 amino acids. It is a member of the lipid transfer lipopolysaccharide binding protein family which facilitates redistribution of cholesteryl ester and triglyceride among lipoproteins[25]. It transfers cholesteryl esters from HDL to apolipoprotein-B containing particles in exchange for triglycerides, thereby reducing the concentration of HDL-cholesterl and increasing non-HDL cholesterol, a lipoprotein distribution predisposing to atheroma formation[26]. The essential role of CETP on human lipoprotein metabolism is evident based on a markedly altered lipoprotein profile in patients with genetic CETP deficiency[27,28]. High plasma levels of CETP are associated with reduced HDL cholesterol levels[261,29-31] and increased LDL cholesterol levels [32]. Several studies have also shown high CETP levels to be atherogenic[33,34.]

In humans, CETP mRNA encodes a polypeptide of Mr 53000, which is n-glycosylated at 4 sites, giving rise to the mature form of CETP of Mr 74000[35.] CETP is expressed primarily in liver, spleen, and adipose tissue, and lower levels have been detected in the small intestine, adrenal gland, heart, kidney, and skeletal muscle [35,36]. There is a wide variation in plasma CETP activity within and between population groups.

The gene for human CETP contains 25 kb genomic DNA and is composed of 16 exons. It has been localized on chromosome 16q21 adjacent to the lecithin-cholesterol acyl transferase gene. Several common restriction fragment length polymorphisms (RFLPs) have been reported in the CETP gene locus [37,38.]

TaqIB polymorphism is most widely studied, which is created by a silent base change affecting the 277th nucleotide in the first intron of the CETP gene [37], resulting in two alleles B1 and B2. The CETP TaqIB polymorphism is associated with changes in plasma CETP concentrations, HDL cholesterol, and risk of CAD[39-43.] Individuals with B1 allele have higher CETP mass, CETP activity and low HDL cholesterol than individuals with B2 allele [40]. Most of the reports in Asians have shown a positive association between the B1 allele and CAD [44,45] Due to its intronic location this polymorphism cannot be considered as a part of a functional regulatory site, but can be a marker for another functional site. Its effect on plasma CETP mass and HDL-C can be accounted by its linkage with many 5′ promoter region base changes like −629C/A, −971G/A and −1337C/T polymorphisms [46-48.]

In view of this we have evaluated the distribution of Cholesteryl ester transfer protein gene Taq1B polymorphism and the concerned phonotype (Cholesteryl ester transfer protein activity) was analysed by using a fluorometric assay kit.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Coronary heart disease has been defined as impairment of function of heart due to inadequate blood supply to the heart compared to its needs caused by atherosclerosis. It being a multifactorial disease has a complex etiology. Many genetic and environmental factors act in combination to determine an individual's risk of developing coronary heart disease[4]. A large number of studies such as the Framingham heart study[5], The Lipid research clinic's coronary primary prevention trial, the Helsenki Heart study, have been conducted to examine the role of risk factors for coronary artery disease. The risk factors identified by these epidemiological studies include a group of fixed risk factors like positive family history, age, male gender, and a group of modifiable risk factors like blood lipid profile abnormalities, hypertension, physical inactivity, obesity, cigarette smoking, alcoholism, diabetes mellitus, hyperhomocysteinemia[5.] Though overwhelming evidence particularly that given by response to "Response to retention hypothesis" indicates that the whole sequence of events is found to be initiated by the retention of modified Low Density Lipoprotein[8,9], it was identified later that despite changes in lifestyle and the use of new pharmacologic approaches to lower plasma cholesterol[10,11]. Cardiovascular disease continues to be principal cause of death[12,13] .

ATHEROSCLEROSIS

Atherosclerosis is a disease affecting arterial blood vessels. It is a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells promoted by low density (especially small particle) lipoproteins without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a "hardening" or "furring" of the arteries. It is caused by the formation of multiple plaques within the arteries.

The lesions of atherosclerosis occur principally within the innermost layer of the artery wall, the intima .They include[14 –16],

- Fatty streak
- Fibrous plaque
- Complicated lesions
    - Plaque disruption
    - Atherothrombosis

FATTY STREAK

The process of atherogenesis begins in childhood with the development of fat, lipid rich lesions called fatty streaks. They are also found to contain macrophages, T lymphocytes, smooth muscle cells – each of these cells are found to contain deposits of cholesterol and cholesterol oleate. The lesions are yellowish and sessile in appearance and they cause little or no obstruction of the affected artery and no clinical sequelae. Observations suggest that lipid deposition does not inevitably lead to the advanced lesions of atherosclerosis but that a number of factors are associated with the progression of the lesions and with the development of more complex form of atherosclerosis, the fibrous plaque.

FIBROUS PLAQUE

The fibrous plaques are derived from fatty streaks that continue the process of cell proliferation, lipid accumulation, and connective tissue formation and that the deep core of lipid and cell debris results from inadequate blood supply, inflammation, and cell necrosis. There is a lesion that is accepted as a forerunner of the fibrous plaque – this is known as fibromusculoelastic or intermediate lesion of the intima, which consists of proliferated smooth muscle cells surrounded by connective tissue and contains little or no lipid. A fully

blown fibrous plaque consists of numerous smooth muscle cells surrounded by a dense connective tissue matrix often intermixed with numerous macrophages. This cap covers a deeper layer of macrophages filled with lipid that are often intermixed with variable number of T lymphocytes.

ADVANCED LESIONS – PLAQUE DISRUPTION AND ATHEROTHROMBOSIS

The typical advanced, complicated lesion contains a large necrotic core with a fibrous core, loaded with macrophages. The macrophages can form numerous proteolytic enzymes, including metalloproteinases – these enzymes cause the removal of fibrous cap – thus plaque disruption is found to happen at the shoulder of the lesion where the cap is thin and the concentration of macrophages is the greatest. The plaque disruption allow the lesion to get involved in thrombotic episodes that can lead to occlusive disease[17.]

Thus, atherosclerosis causes two main problems.

1. First, the atheromatous plaques, though long compensated for by artery enlargement, eventually lead to plaque ruptures and stenosis (narrowing) of the artery and, therefore, an insufficient blood supply to the organ it feeds.

2. Second, if the compensating artery enlargement process is excessive, then a net aneurysm results.

These complications are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures, causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes. This catastrophic event is called an infarction.

The clinical scenario of this catastrophic event depends on which artery is affected by this event.

1. One of the most common recognized scenarios is thrombosis of a coronary artery, causing myocardial infarction (a heart attack). This condition is called as Coronary artery disease.

2. Second most common is that caused due to thrombosis of carotid artery branches and inadequate blood supply to brain – which presents itself as stroke or transient ischemic attack.

3. Another common scenario in very advanced disease is claudication from insufficient blood supply to the legs, typically due to a combination of both stenosis and aneurysmal segments narrowed with clots. This is called as peripheral artery disease.

4. Since atherosclerosis is a body-wide process, similar events also occur in the arteries of the intestines, kidneys, legs, etc.

HYPOTHESIS OF ATHEROGENESIS

Atherosclerosis has been recognized in humans for thousands of years. Long has discussed the development of clinic-pathologic correlations that evolved during the era when autopsy examination permitted the formulation of a hypothesis relating the degree of atherosclerosis to the incidence of myocardial infarction and stroke[13]. Virchow proposed the idea that some form of injury to the arterial wall associated with the inflammatory response resulted in the degenerative lesion of atherosclerosis[18]. This idea was subsequently modified by Antischkow[19] and further included the role of platelets and thrombogenesis in

atherosclerosis as expanded by Duguid[16]. John French noted that the structural integrity of the endothelial lining[20] of the artery represented a key element in the maintenance of normal arterial function and that alteration in endothelial integrity might precede a sequence of events that would lead to the various forms of the lesions of atherosclerosis.

RESPONSE TO INJURY HYPOTHESIS

In normal artery, the endothelial cells form a continuous monolayer that regulates the passage of substances from the plasma to the underlying wall[49-51], forms a thromboresistance surface that promotes the continuous flow of blood throughout the vascular tree[52,53], by production of certain cytokines regulates the migration and proliferation of smooth muscle cells and they are metabolically active. Endothelial cells are capable of transporting plasma lipoproteins of given sizes into the arterial wall[54]. The endothelium exhibits the thromboresistant characters by production of three factors. They are the cell surface glycoproteins and proteoglycans that form the surface coat of the endothelial cells, prostacyclin[52], and the most potent agent, nitric oxide[53]. Prostacyclin and NO are potent vasodilatory agents and potent inhibitors of platelet aggregation. The hypothesis posits that some form of "injury" to the endothelium results in structural and functional alterations in the endothelial cells, in such a way that they would permit plasma constituents such as lipoproteins and inflammatory cells to have a more ready access to the artery wall.

The endothelial dysfunction is associated with overexpression of E, L, P selectin that appear to play a role in inducing rolling and attachment of monocytes and T lymphocytes to endothelium. This rolling is facilitated by the upregulation of ICAM 1 and VCAM 1 also. Another molecule formed by endothelium, PeCAM 1 has been shown to participate in interendothelial migration by the adherent leukocyte into the subendothelial space or intima of the artery. Thus, the earliest phase of the chronic, inflammatory response that has become

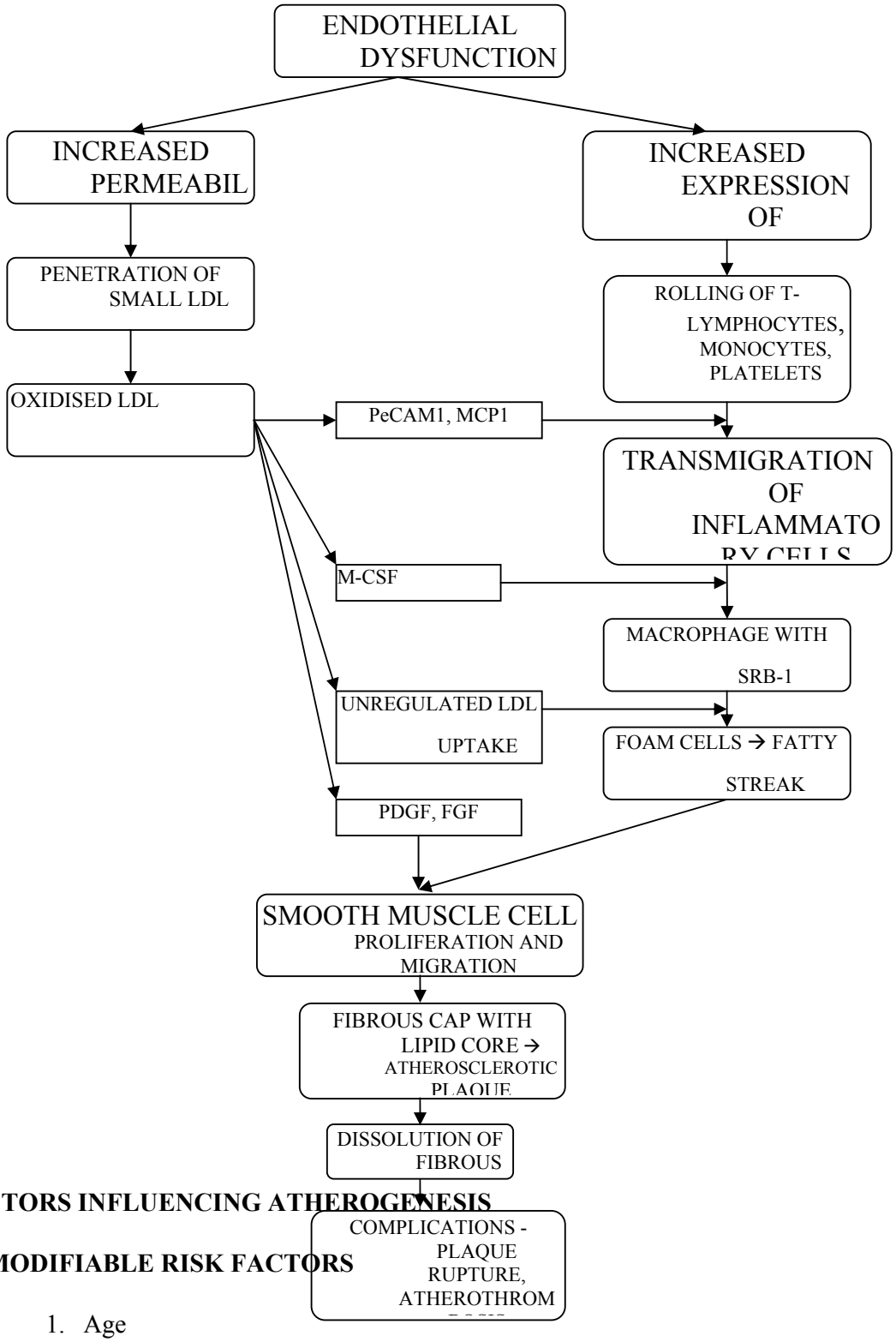
recognized to be the hallmark of atherogenesis is represented by leukocyte adhesion due to the formation of these attachment and adherence molecules on the surfaces of the endothelium and the leukocytes[55-57].

A second event accompanying endothelial dysfunction is transmigration of lipoproteins particularly of small LDL particles, this transmigration places LDL in the subendothelial space which is virtually devoid of any antioxidant properties of the circulation, hence it gets oxidised. Oxidised LDL can act as one of the chemotactic reagents and can also induce the endothelial cells and the underlying smooth muscle cells to form a second chemotactic factor, monocyte chemoattractant protein 1 (MCP-1) and a colony stimulating factor (M-CSF). In this scenario, the monocyte gets activated to macrophages, which express SR-B1 causing unregulated uptake of LDL particles, forming foam cells. Such a lesion with foam cells, activated inflammatory cells is called as fatty streak.

Oxidised LDL, foam cells, the activated macrophages, T-cells, produce various cytokines IL-1, TNF-α. Under the influence of these cytokines, endothelium, macrophages and T cells produce PDGF, FGF[58]. PDGF stimulates smooth muscle cell migration and proliferation. FGF stimulate the vascular smooth muscle cell to produce collagen and the various components of extracellular matrix together they form the fibrous cap. TNF-α induces apoptosis of foam cells causing exocytosis of its lipid content, which forms the lipid core. Such a lesion with lipid core, surrounded by activated T-cells, macrophages, platelets (occasionally), lined by a fibrous cap is called as a stable atherosclerotic plaque. Thus oxidised LDL is not only toxic to the endothelium and the surrounding cells in the intima but also chemotactic for monocytes and can activate monocyte derived macrophages to produce growth factors and cytokines. Hence it may be the principal culprit in advancing the lesions of atherosclerosis.

If the injury to the endothelium were a self-limited event and if endothelial function were restored, the proliferative lesions might be capable of regressing. If this were the case, the lesions would be reversible and, if they had not reached a critical size, would be clinically silent. On the other hand, if the injury at focal sites in the artery wall is either of long standing or chronically repeated over period of many years, the lesion could continue to progress, becoming increasingly complex in terms of their composition. Because, the atherosclerotic plaque does not only have smooth muscle cells but also macrophages, which are capable of producing metalloproteinases and TNFα, both of which cause necrosis and digestion of the fibrous cap, this loss of fibrous cap is responsible for the complications of atherosclerosis, namely plaque rupture. This plaque rupture exposes the subendothelial extracellular matrix to the factors of coagulation in the circulation initiating the intrinsic pathway of coagulation – this is responsible for atherothrombosis.

Fig 1. PATHOGENESIS OF ATHEROSCLEROSIS



FACTORS INFLUENCING ATHEROGENESIS

UNMODIFIABLE RISK FACTORS

1. Age
2. Male sex

3. Socioeconomic status

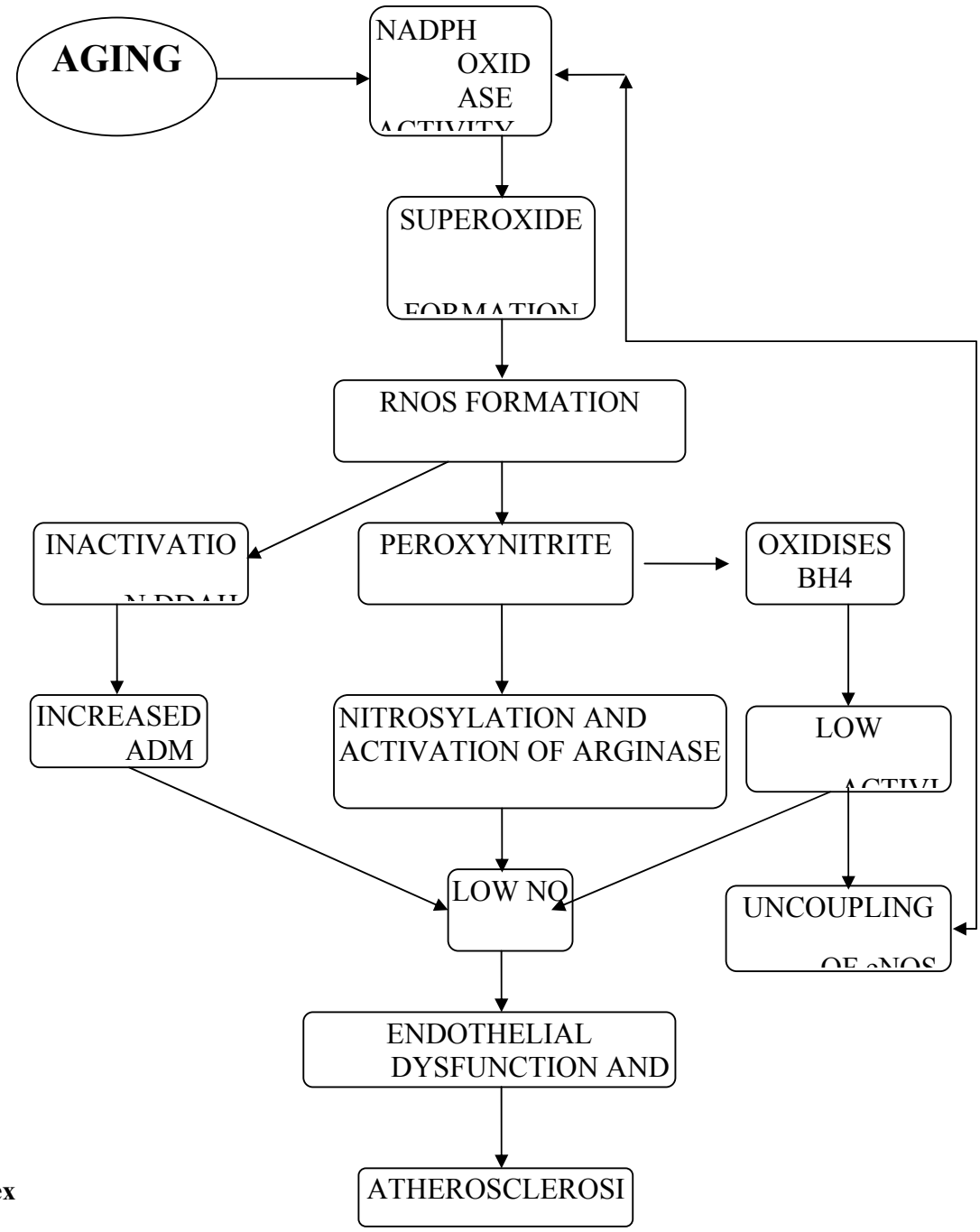
MODIFIABLE RISK FACTORS

1. Cigarette smoking
2. Alcoholism
3. Insulin resistance & hyperglycemia
4. Hypertension
5. Obesity
6. Oxidative stress
7. Abnormal lipid profile
    a. High total and LDL cholesterol
    b. Low HDL cholesterol
    c. High triglycerides
    d. Lipoprotein(a)
8. Physical inactivity

Age

In many epidemiologic surveys, age remains one of the strongest predictors of disease. The majority of patients with atherosclerotic coronary heart disease are more than 65 years old. Older patients have higher mortality and more complications. Age related changes in the cardiovascular system and other organs make it reasonable to assume that aging per se constitutes a major reason for the increased morbidity and mortality in older persons. These age related changes include diastolic dysfunction, degenerative changes in the conduction system, reduced responses to catecholamine and sympathetic stimuli.

Fig 2 ROLE OF AGING IN ATHEROSCLEROSIS



Male sex

The relationship of gender to the development and prognosis of atherosclerotic coronary heart disease is complicated[59]. The powerful protective effect of the premenopausal

state in preventing and postponing the condition is fully appreciated; women tend to develop atherosclerotic coronary heart disease approximately 10 years later than men. Typical and atypical symptoms of angina pectoris are less likely to be associated with significant epicardial coronary atherosclerosis in women especially in those under the age of 60[59,60]. Complications are fewer in women after the onset of angina, but they may be more frequent after myocardial infarction[61,62]. However there are multiple reports indicating a gender bias in reference to the use of diagnostic and therapeutic procedures, but interpretation is complicated by the possibility of overuse and overtreatment in low risk men[63-67]. The point has been clearly made however; atherosclerotic coronary heart disease manifest as angina, infarct, and sudden death is as common in women after age 60 as it is in men.

This gender dependent differential risk is attributed to the protective function exerted by estrogens. Recently, a study of estrogens and their effect on smooth muscle cells and the other elements of atherogenesis showed that estrogens have an antiproliferative effect on smooth muscle cells and can be protective to the endothelium in relation to stimulation by growth factors, cytokines and other agents. Estrogen is not only antiproliferative for smooth muscle but also has been shown to be capable of modulating acetylcholine mediated dilation of atherosclerotic coronary arteries[68].

Family history of Early- Onset CHD

Over 35 case-control and prospective studies have consistently identified an association between CHD and a history of first degree relatives with early onset CHD[69]. This risk generally persists even after adjustment for other risk factors. The family history most predictive of coronary disease is that of a first degree relative developing CHD at an early age. Although CHD in a male relative with onset at age 55 or less or a female relative with onset at age 65 or less is defined as a positive family history[70], the larger the number of

relatives with early onset of CHD or the younger the age of CHD onset in the relative, the stronger the predictive value [71,72].

Socioeconomic status

A consistent relationship has been devised between lower socioeconomic status and atherosclerosis. There has been the perception that conventional risk factors cluster in lower socioeconomic groups and that this phenomenon can explain the increased incidence of atherosclerotic coronary heart disease[73]. But, only 50% of atherosclerotic coronary heart disease can be explained by known risk factors. The socioeconomic status proved to be independent predictors in patients with established atherosclerotic coronary heart disease[74]. Although no simple relationship between socioeconomic status, risk for cardiovascular disease and long term outcome for manifest atherosclerotic coronary heart disease can be devised, the evident is consistent and persuasive that lower socioeconomic status is an independent and significant determinant of long-term outcome.

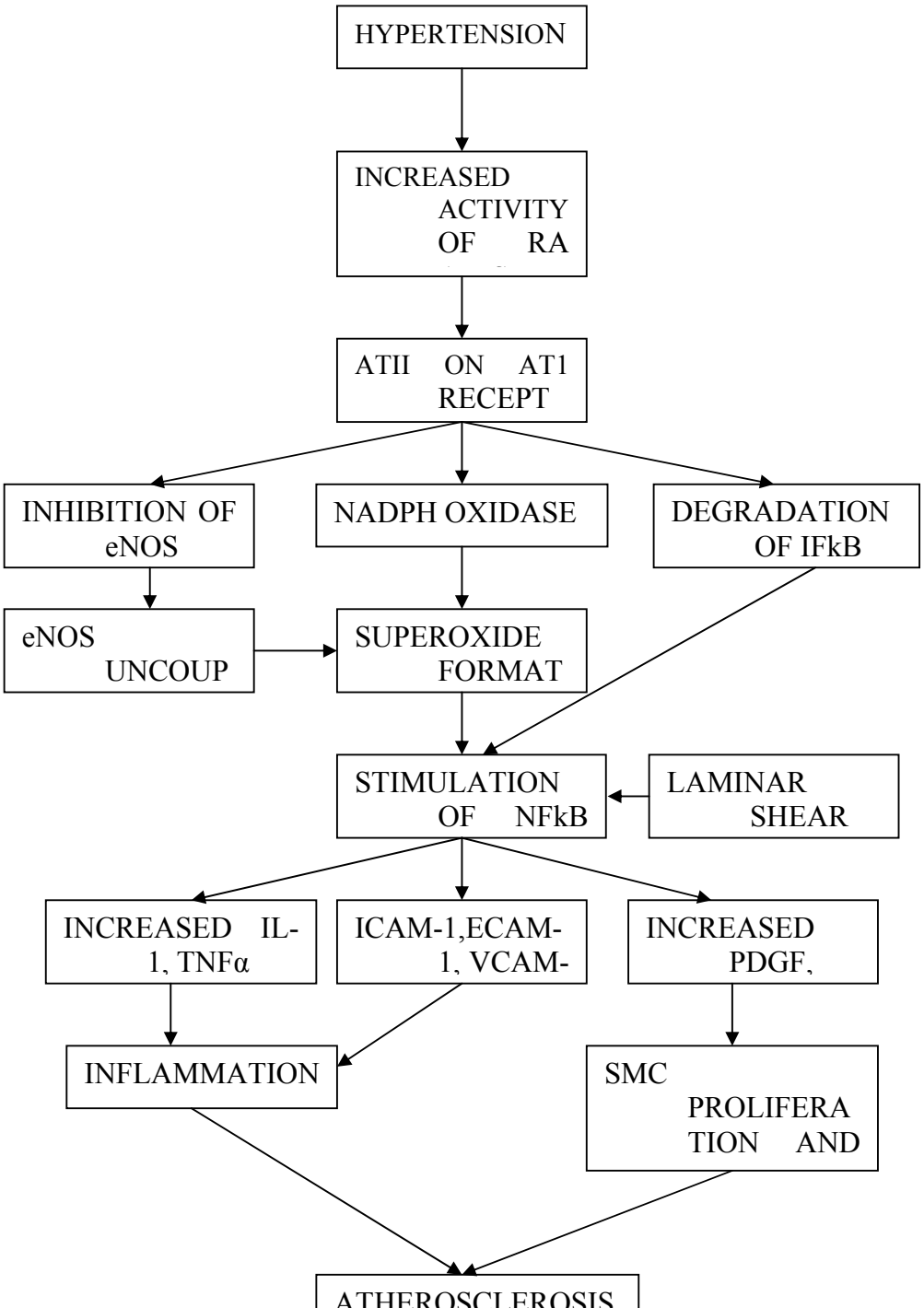
Hypertension

Several major prospective epidemiological studies have found that both systolic and diastolic hypertension have a strong, positive and graded relationship to CHD without evidence level of a threshold risk level of blood pressure[75-77]. The risk imposed by hypertension is heightened substantially when other risk factors are present. Hypertension clusters with insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia, left ventricular hypertrophy and obesity and occurs in isolation in fewer than 20% of individuals[78.]

The potential mechanisms by which hypertension may cause impaired endothelial function include increased endothelial permeability to lipoproteins, increased adherence of

leukocytes, increased oxidative stress, and hemodynamic stress that may trigger acute plaque rupture, all these mediated by activation of NF-kB pathway and inactivation of eNOS enzyme.

Fig 3. EFFECT OF HYPERTENSION ON ATHEROSCLEROSIS

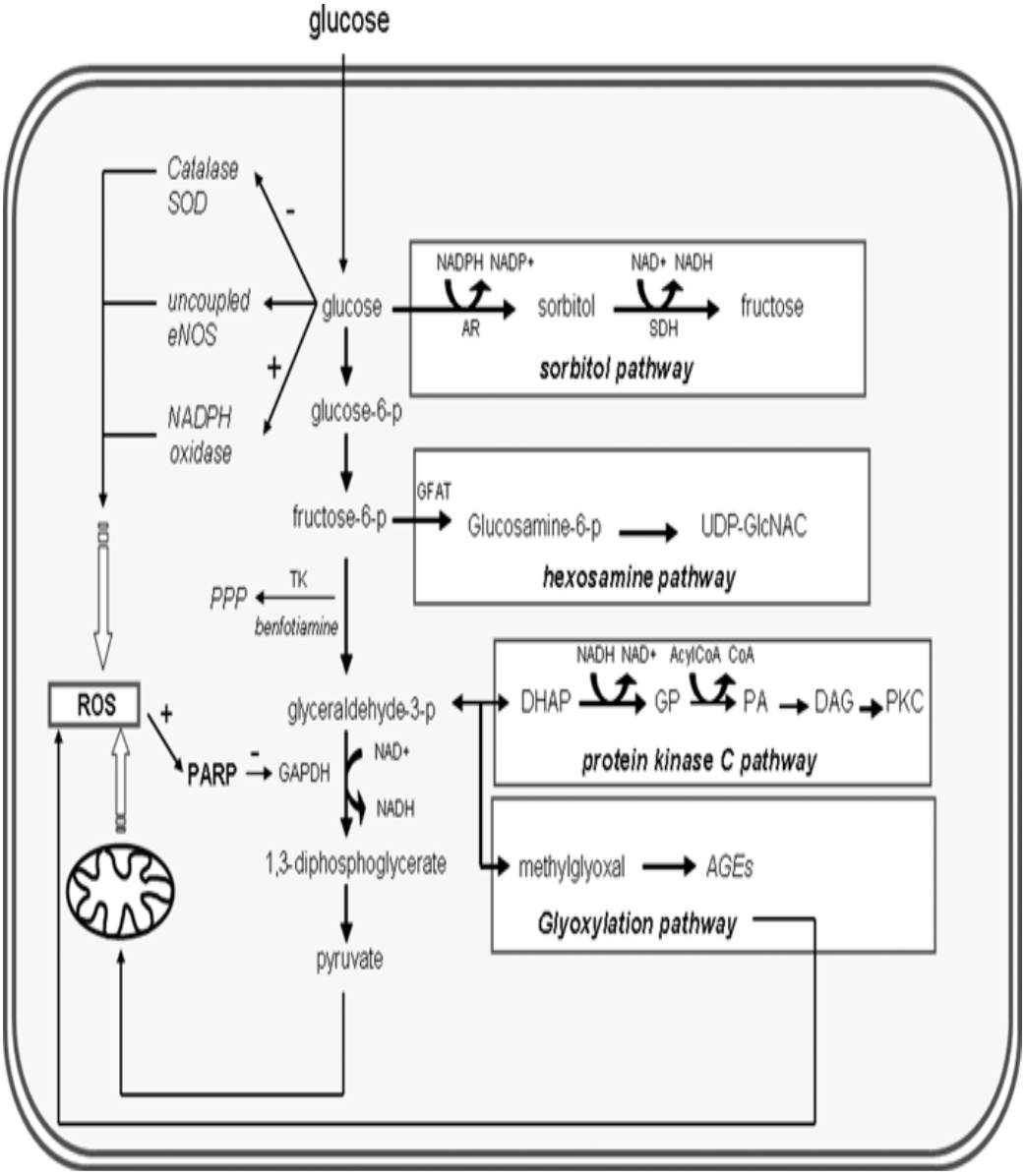


Hyperglycemia

Hyperglycemia is an independent risk factor for CHD, increasing the risk by two to three times for men and three to five times for women[79]. CHD is the leading cause of death in diabetic patients and approximately 25% of MI survivals have diabetes [80.] The CHD risk for a premenopausal diabetic woman is similar to the risk of a nondiabetic man, hence diabetes abolishes the protective effect of being a premenopausal female[81]. Diabetic women have twice the risk of recurrent MI compared with diabetic men[82]. The greater risk of CHD in diabetic women compared to diabetic men may be explained in part by the greater adverse effect of diabetes on lipoproteins in women [83].

Potential mechanisms by which hyperglycemia may cause atherosclerosis include impaired endothelial function, glycation of LDL, enhanced lipoprotein oxidation, increased fibrinogen, increased platelet aggregation, increased PAI-1, impaired fibrinolysis, increased small LDL. All these are attributed to the increased flux of glucose into glycolysis (glucose uptake and hexokinase activity in endothelium is insulin independent), as a result there is an increased NADH/NAD ratio, causing increased flux of electrons through electron transport chain, producing superoxide radicals. This cause DNA damage and the resultant ADP ribosylation of proteins inhibit glyceraldehyde 3-phosphate dehydrogenase of glycolysis, causing accumulation of glyceraldehyde 3- phosphate and its precursor fructose 6-phosphate. The former cause activation of protein kinase C pathway through DAG – protein kinase C pathway stimulates the production of various cytokines and thereby stimulates inflammation. Fructose 6 phosphate stimulates hexosamine pathway, thereby stimulate N- glycosylation of many proteins like eNOS and inhibition of them, this causes NOS uncoupling and the resultant oxidative stress further aggravates the condition. Furthermore, there is increased formation of advanced glycation end product, which on binding to receptor for advanced glycation end products is found to stimulate NF-kB pathway, causing all the features of atherosclerosis.

Fig 4. PATHOGENESIS OF ATHEROSCLEROSIS IN DIABETES MELLITUS



Insulin resistance and hyperinsulinemia

Resistance to insulin and compensatory hyperinsulinemia are the common metabolic basis of cluster of coronary risk factors, particularly hypertension, diabetes, hypertriglyceridemia, low HDL, predominance of small LDL, and an increased plasminogen activator inhibitor concentration[84,85]. Hyperinsulinemia may raise blood pressure through sympathetic nerve stimulation and/or renal sodium retention. Insulin sensitivity is associated with endothelial nitric oxide production in healthy persons providing a clue as to how insulin resistance may promote CHD directly[86]. Furthermore, hyperinsulinemia has been found in a prospective study to be an independent risk factor for CHD in nondiabetic men after adjusting for body weight, blood pressure and dyslipidemia[87].

Physical inactivity

Physical inactivity roughly doubles the risk of CHD. Moderate intensity exercise reduces coronary atherosclerosis and widens coronary arteries in monkeys fed on atherogenic diet compared with monkeys fed the same diet but forced to be sedentary[88]. Physical activity slows progression of angiographically defined coronary atherosclerosis in human[89]. Over 50 obsevational studies, primarily of men, have established that physical fitness, on the job of physical activity, and leisure time physical activity reduce the risk of CHD[90]. Higher levels of physical fitness and leisure time physical activity are associated with lower rates of mortality, independent of other risk factors[90]. The risk of MI and sudden cardiac death is greatest during exercise, leading some to question the benefits of exercise[91]. The overall risk of myocardial infarction and sudden cardiac death, is however low among those who exercise regularly. The greatest reduction in risk is between sedentary individuals and those who do regular moderate intensity activity.

In addition to decreasing myocardial oxygen demand and increasing myocardial efficiency and electrical stability, other potential mechanisms by which physical activity may reduce CHD risk include increasing HDL, reducing blood pressure, reducing obesity, improving insulin sensitivity, decreasing platelet aggregation and increasing fibrinolysis[90].

Obesity

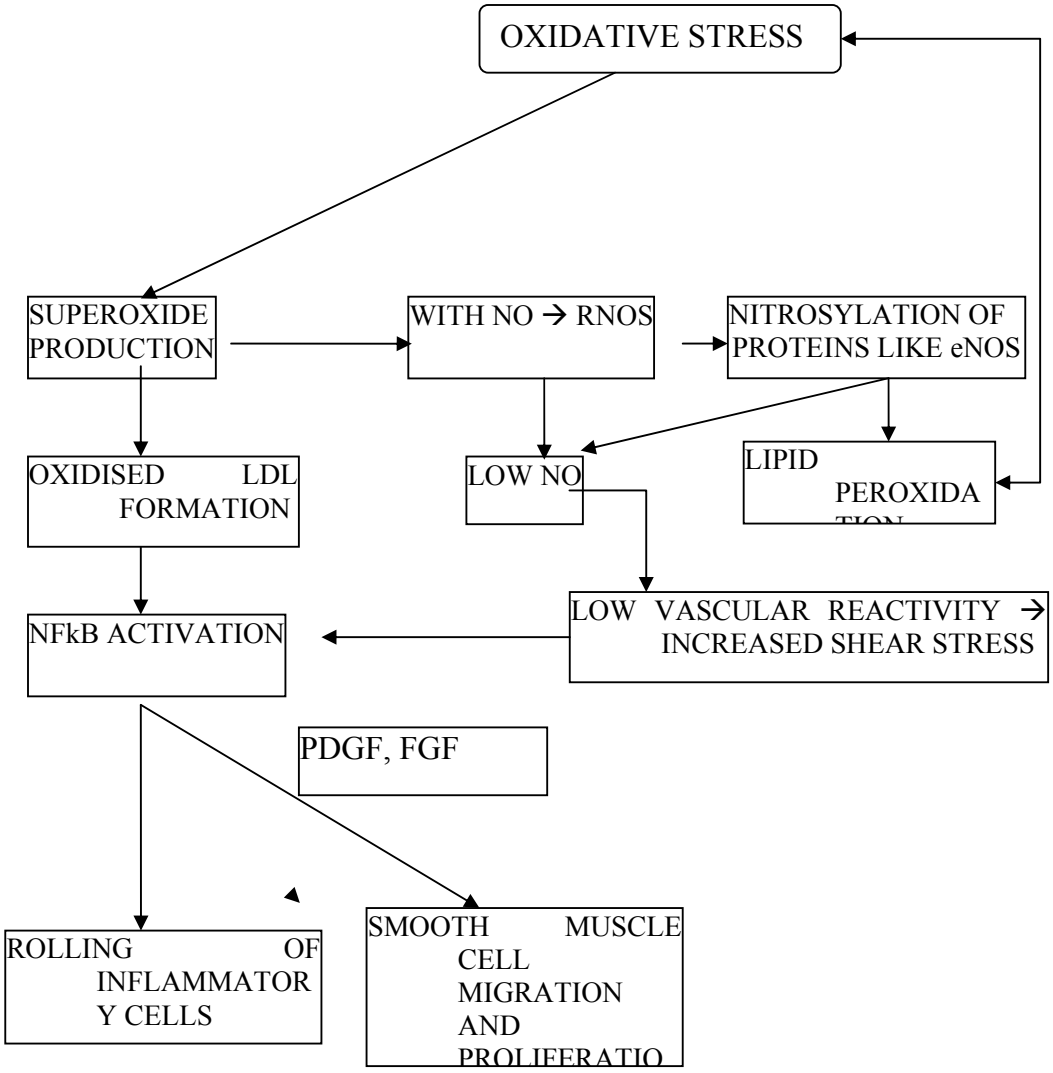
Obesity promotes insulin resistance, hyperinsulinemia, hypertriglyceridemia, low HDL cholesterol, and LVH [92,93]. Many observational studies have found that obesity strongly and positively correlates with the risk of CHD in univariate analysis. In multivariate analysis, when controlling statistically for risk factors such as hypertension, diabetes, and dyslipidemia, obesity is not found to be an independent risk factor. Rather it reflects that much of the adverse consequences of obesity are mediated through resultant metabolic risk factors acting as pathological links in the causal pathway. Nevertheless, some large prospective observational studies of long duration indicate that obesity is an independent risk factor for coronary and cardiovascular mortality in men and women[94-96]. The central distribution of body fat predicts CHD in men independently of body-mass index and other major risk factors[97]. Weight loss improves insulin sensitivity and glucose disposal; reduces blood pressure, triglycerides and LVH; and increases HDL cholesterol[92,93].

Oxidative stress

Excessive production of reactive oxygen species has been implicated to play an important role in a number of cardiovascular pathologies, including hypertension, atherosclerosis, myocardial infarction, ischemia/reperfusion injury. ROS are generated in vascular cells by NADPH oxidases, uncoupled eNOS, and other enzymatic sources or as a product of mitochondrial respiration[59]. If this production goes unbalanced, it leads to

exacerbation of pathophysiological processes. Superoxide radicals are found to cause oxidative modification of LDL. Oxidised LDL, by activating NF-kB pathway of inflammation is found to mediate the increased production of IL-1, increased expression of ICAM, both of which mediate the rolling of inflammatory cells. Furthermore there is increased production of PDGF and FGF, both of which cause smooth muscle cell proliferation and migration. This cycle is reinforced by the decreased production of NO, because the superoxide cause nitrosylation of eNOS and thereby it inhibits the enzyme activity. The decreased NO causes increased vascular reactivity and the resultant shear stress will further stimulate NF-kB pathway. Thus oxidised LDL is hypothesized to play a major role in the initiation and progression of atherosclerosis[98-101].

Fig 5. ROLE OF OXIDATIVE STRESS IN ATHEROSCLEROSIS



Cigarette smoking

Strong dose relationships between cigarette smoking and coronary heart disease have been observed in both sexes. Cigarette smoking increases the risk two to threefold and interacts with other risk factors to multiply risk. Pathophysiological studies have identified a panoply of mechanisms through which cigarette smoking may cause CHD. Smokers have increased levels of oxidation products, including oxidised LDL. Cigarette smoking also lowers the cardioprotective levels of HDL. These effects, along with direct effects of carbon monoxide and nicotine, produce endothelial damage. Possibly, through these mechanisms, smokers have increased vascular reactivity[102]. Cigarette smoking is also related to increased levels of fibrinogen[103] and increased platelet aggregability[104]. Thus cigarette smoking paves way for atherosclerosis by inducing oxidative stress and by altering coagulability.

Dyslipidemia

Total cholesterol and LDL cholesterol

Numerous prospective studies have identified a continuous, graded and direct relationship between serum cholesterol and CHD incidence[105]. The level of total and LDL cholesterol interacts with other risk factors to multiply risk[106]. Elevated LDL cholesterol levels have been related to recurrent events and CHD death in patients with established CHD [107]. Elevated LDL cholesterol levels appear to be involved with all stages of atherogenesis – endothelial dysfunction, plaque formation and growth, and plaque instability and disruption. Elevated cholesterol levels in the plasma lead to an increased retention of LDL particles in the arterial wall, their oxidation and the secretion of various inflammatory mediators and chemoattractants[108]. LDL is also a potent mitogen for smooth muscle cells; progressive growth of atherosclerotic plaques can be halted by lowering of LDL cholesterol levels.

Atherosclerotic plaques with a large lipid core and numerous lipid filled macrophages are prone to rupture[109]. Thus the epidemiological evidence strongly supports LDL-cholesterol's role in atherosclerosis.

Furthermore, small dense LDL is felt to be more atherogenic[110]. Possible explanation for this is that when a person has more of small LDL particles, for given cholesterol content, the number of LDL particles will be more, and an LDL receptor can accept only one LDL particle at a time and hence the rate of metabolism of LDL is decreased, causing accumulation of LDL in the plasma. The second reason for the same is the endothelium will be more permeable to small LDL particle when compared to a normal LDL.

Triglycerides

The relationship between triglycerides and CHD has been less clear. In men, univariate analysis has consistently demonstrated a direct dose-response relationship. This relationship usually disappears after adjustment for other risk factors such as HDL cholesterol, obesity, and diabetes[111]. Hypertriglyceridemia however has been found to be an independent risk factor in women[112]. Several mechanisms have been proposed to explain the triglyceride- CHD association. First, some patients with hypertriglyceridemia have a predominance of small, dense LDL particles. Second, fasting hypertriglyceridemia may be a marker of exaggerated postprandial hyperlipidemia, which may promote the uptake of atherogenic triglyceride rich lipoprotein remnants by endothelial cells[113]. Finally, serum triglyceride levels are strongly related to fibrinogen and factor VII in numerous epidemiological studies[114]. Therefore, number of mechanisms, direct and indirect link serum triglycerides and CHD.

Low HDL cholesterol

Numerous prospective epidemiological studies have demonstrated a continuous, inverse relationship between HDL cholesterol levels and the incidence of CHD[115]. The total cholesterol to HDL cholesterol ratio is a better predictor of CHD than the HDL cholesterol level alone[115]. Two important mechanisms by which HDL is thought to play a protective role against atherosclerosis are reverse cholesterol transport and inhibition of LDL oxidation.

Reverse cholesterol transport

The key role of HDL as a carrier of excess cellular cholesterol in the reverse cholesterol transport pathway is believed to provide protection against atherosclerosis. In reverse cholesterol transport, peripheral tissues (e.g., vessel-wall macrophages) remove their excess cholesterol through the ATP-binding cassette transporter-1 (ABCA1) to poorly lipidated apolipoprotein A-I, forming pre-beta-HDL. Lecithin-cholesterol acyltransferase then esterifies free cholesterol to cholesteryl esters, converting pre-beta-HDL to mature spherical alpha-HDL. HDL cholesterol is transported to the liver by two pathways. Through the first pathway, it is delivered directly to the liver through interaction with the scavenger receptor, class B, type I (SR-BI). Through the second pathway, cholesteryl esters in HDL are transfered by the cholesteryl ester transfer protein (CETP) to very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and are then returned to the liver through the LDL receptor. HDL cholesterol that is taken up by the liver is then excreted in the form of bile acids and cholesterol, completing the process of reverse cholesterol transport.

Cholesteryl ester transfer protein is a plasma glycoprotein that plays an important role in reverse cholesterol transport. It transfers cholesteryl esters from HDL to LDL and VLDL particles in exchange for TG, thereby reducing the concentration of HDL cholesterol and

increasing non-HDL cholesterol, a lipoprotein distribution predisposing to atheroma formation.

Direct proof of a role for cholesteryl ester transfer protein in the development of atherosclerosis has been provided in mouse models. Marotti et al[116] demonstrated that transgenic mice expressing CETP had much worse atherosclerosis than did non-expressing controls, and they suggested that the increase in lesion severity was due largely to CETP-induced alterations in the lipoprotein profile.

CHOLESTERYL ESTER TRANSFER PROTEIN

Plasma cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein, with an M_r of 70,000 to 74,000 [35]. It belongs to a family of proteins that engage in lipid binding and lipid transfer [36]. CETP is secreted mainly from the liver and that circulates in plasma, bound mainly to HDL [29]. Analysis of human plasma by gel filtration or apolipoprotein A-I immunoaffinity chromatography indicates that the major part (> 80%) of CETP is associated with HDL particles. Although CETP binds with similar affinity to VLDL, LDL, and HDL, the molar concentration of plasma HDL is much higher than that of plasma VLDL or LDL, explaining the predominant association with HDL in plasma [117]. CETP dissociates from lipoproteins during ultracentrifugation.

CETP promotes the redistribution of cholesteryl esters, triglycerides, and, to a lesser extent, phospholipids between plasma lipoproteins. CETP transfers lipids from one lipoprotein particle to another in a process that results in equilibration of lipids between lipoprotein fractions. CETP contributes to an atherogenic lipid phenotype in several ways. It increases the cholesteryl ester content and thus, the atherogenicity of VLDL and LDL. The

CETP-mediated reduction in HDL particle size is accompanied by the dissociation of lipid-poor apolipoprotein A-I (apoA-I) from the particle.

STRUCTURE OF CHOLESTERYL ESTER TRANSFER PROTEIN

Human CETP consists of 476 amino acid residues. The amino acid analysis of purified CETP showed an unusually high content (44%) of non-polar residues. The CETP complementary DNAs (cDNAs) were cloned from humans, rabbits, cynomolgus monkeys and hamster. Human CETP cDNA has an approximately 80% homology to that of rabbits.

CETP belongs to a family of proteins that engage in lipid binding (lipopolysaccharide-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI)) and lipid transfer (CETP and PLTP) [36], but emerging family members may have other functions [118.] Although these four proteins possess different physiological functions, they share marked biochemical similarities. All of these four proteins can bind lipopolysaccharides and phospholipids as well as a variety of other lipids. CETP, PLTP and LBP are associated with plasma HDL; however, BPI exists on the membranes of secretory granules of neutrophils. BPI is a long boomerang-shaped molecule having two domains at the NH2- and COOH-terminus with similar folds shaped like barrels. BPI contains a central beta -sheet domain which forms an interface between the barrels. Each barrel has a pocket occupied by a phosphatidylcholine molecule. CETP may have a similar structure.

28% of molecular mass of CETP is attributed to N-glycosylation at residues 88, 240, 341 and 396[119] The high content of glycosylation, hydrophobic residues and free cysteines (five) presented significant challenges for structural studies.

CETP has an elongated ‘boomerang’ shape with dimensions of 135 A° X 30 A° X 35 A° and a fold homologous to that of BPI[118]. The fold consists of two similar domains

connected by a linker, residues 240–259 in CETP (Fig. 6a). The structure of CETP can be divided into four structural units: one barrel at each end of the protein (barrels N and C), a central beta-sheet between the two barrels and a C-terminal extension that is not present in BPI (Fig. 6b). Each barrel contains a highly twisted beta-sheet and two helices (A and B in barrel N, A′ and B′ in barrel C), with helices B and B′ being longer than A and A′. The central beta-sheet includes six antiparallel strands consisting of residues before and after the barrels. These three structural units in CETP overlay well with the homologous units in BPI. The fourth unit, Glu465–Ser476 at the C terminus of CETP, forms a distorted amphipathic helix, helix X that unwinds slightly at the end.

The structure of CETP reveals four bound lipid molecules, which must have been incorporated during protein production, as no lipid was introduced after expression. CETP has two neutral lipid– binding sites and two phospholipid-binding sites. The four lipids occupy a 60-A°-long, continuous tunnel that traverses the core of the protein. The CETP tunnel is formed by a wall of beta-sheets underneath the bound lipids and a layer of helices above the lipids (Fig. 6b). The CETP concave surface is the site of the N and C openings, helix X and the Ω1 flap, making this surface the most likely to bind lipoproteins (Fig. 6a). The surface contains numerous charged and hydrophobic residues that are distributed evenly rather than in distinct patterns, suggesting evenly distributed interactions with lipoprotein surfaces. The sheer size of the interface and the apparent lack of a ‘hot spot’ suggest that small alterations of the concave surface should not greatly change lipoprotein affinity and explain the lack of detectable activity changes in concave-surface mutants.

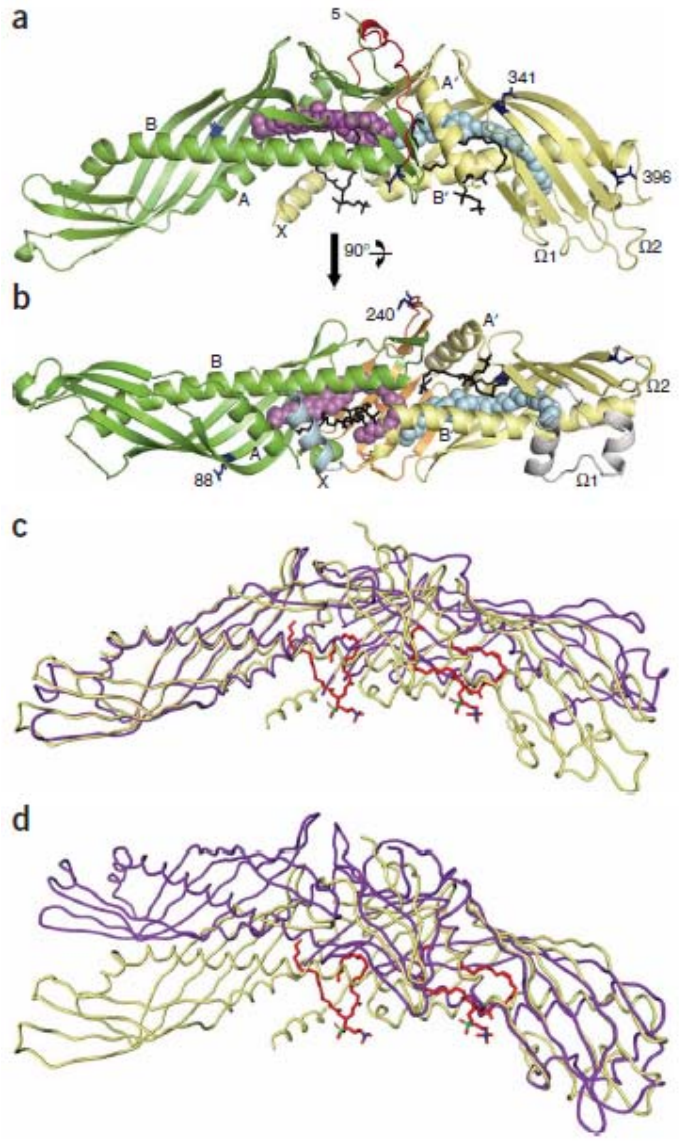


Figure 6 : **Overall structure of CETP**. (a) Ribbon diagram of N-terminal (green) and C-terminal (yellow) domains, with linker in red. N-terminal side is shown on left. CE1 (magenta) and CE2 (cyan) are shown as space fills and phospholipid as black bonds. N-glycosylation sites are shown as blue bonds, with 341 and 396 labelled. '5' marks the observed N terminus. Helices A, B, A□, B□ and X are labelled. Helix X belongs to the C-terminal domain but interacts with residues of the N-terminal domain. (b) The view after a 90° rotation. The four structural units shown are barrel N (green), central beta-sheet (orange), barrel C (yellow) and helix X (cyan). The Ω1 flap is in grey. N-glycosylation sites 88 and 240

are labelled. (c, d) Cα traces of CETP (yellow) and BPI (purple) with the barrel Ns (c) or barrel Cs (d) overlaid. CETP-bound phospholipid molecules are in red.

SYNTHESIS OF CHOLESTERYL ESTER TRANSFER PROTEIN

CETP is synthesized by the liver, spleen, small intestine, adipose tissues, adrenal gland, kidney, heart, and skeletal muscles [35,36]. CETP is secreted by a variety of cell types, including monocyte-derived macrophages, B-lymphocytes, adipocytes, hepatocytes and a human hepatoma cell line, HepG2 cells, and CaCo-2 cells [29]. In cynomolgus monkeys, high levels of CETP mRNA were shown in the liver and thoracic aorta. Tissues containing LPL such as adipose tissues and skeletal muscles were shown to be the major sources of CETP mRNA in hamsters. There is a marked species difference in CETP activity. CETP activity is very low in mice and rats and high in rabbits. The CETP activity in humans is intermediate between mice and rabbits.

Human cultured monocyte-derived macrophages synthesize and secrete CETP activity. Induction of CETP expression occurs during the differentiation of monocytes into macrophages. The secretion of CETP activity into culture medium is facilitated by cholesterol loading with acetylated LDL or free cholesterol. The intracellular accumulation of cholesteryl ester is positively correlated with the secretion of CETP, suggesting that CETP may function to maintain intracellular cholesterol homeostasis during differentiation and in response to an excess of cholesterol accumulation. CETP mRNA abundance in human adipose tissue was demonstrated to be a function of membrane cholesterol content rather than lipid droplet cholesterol.

CETP activity is also detected in seminal fluid and cerebrospinal fluid, which is about 12% of that of plasma [120.] CETP activity can be detected in the conditioned medium from

human neuroblastoma and neuroglioma cells and from sheep choroid plexus. Therefore, it is suggested that CETP may play some roles in the transport and redistribution of lipids within the central nervous system and adipocytes.

ALTERNATIVE SPLICING OF CETP mRNA

Two isoforms (Figure:7-A and B) of CETP mRNA were demonstrated in human tissues expressing the CETP gene; one was a full-length form which produces an active Mr 74 000 CETP, and the other a shorter variant in which exon 9-derived sequences had been removed [121.] The exon 9-deleted form of CETP mRNA is produced by alternative splicing of the CETP gene transcript. Exon 9 contains 180 nucleotides within the amino acid encoding sequence of the CETP mRNA. The splicing of exon 9 results in the removal of 60 amino acids from the CETP sequence, without alteration of the translational reading frame. The alternatively spliced CETP mRNA is present in all human tissues containing the CETP mRNA, but its abundance varies from about 20% of total CETP mRNA in liver to 40-60% in spleen.

By transfection of the exon 9-deleted cDNA to COS (CV-1 origin, SV-40) cells, a shortened, poorly secreted and inactive form of CETP was produced. When full-length and exon 9-deleted cDNAs were co-transfected to COS cells, the secretion of full-length active CETP was inhibited. Therefore, the exon 9-deleted protein may have a dominant negative effect on the expression of full-length CETP and alternative splicing of the CETP gene may modulate the level of active CETP.

The Caco-2 (colorectal adenocarcinoma) cells, a model cell line of intestines, show a low rate of active CETP mRNA under basal conditions and about 60% of reverse transcribed CETP cDNA corresponds to exon 9-deleted transcripts. However, addition of sodium oleate

into the culture medium induces a 2-fold increase in full-length CETP cDNA transcripts without affecting exon 9-deleted transcripts, resulting in an enhancement of CETP activity secreted into the medium. Thus, alternative splicing of the human CETP gene in each tissue may modulate the expression of active CETP.

STRUCTURE FUNCTION RELATIONSHIP OF CETP

The epitope of a neutralizing monoclonal antibody was shown to be the carboxyl (C)-terminal sequences of CETP (26 amino acids), which are important for neutral lipid transfer activity [122]. Some monoclonal antibodies completely inhibited triglyceride transfer, but not cholesteryl ester transfer, suggesting that the binding sites of cholesteryl ester and triglyceride may be different. The C-terminal sequence -Phe-Leu-Leu-Leu- (residues 454-457) was essential for normal binding of CETP to each lipoprotein and the effective transfer of cholesteryl ester and triglyceride, possibly together with the other sequences in the C-terminal region. Interestingly, a similar conserved sequence exists in several other proteins that function in binding non-polar lipids, including LCAT, cholesterol 7α - hydroxylase, cholesterol esterase, and hormone-sensitive lipase. The Mr of CETP polypeptide deduced from the cDNA is 53108; however, purified plasma CETP appears as a broad band containing two different molecular forms. This micro-heterogeneity could be explained by the variable N-linked glycosylation.

FIG : 7 COMPARISON OF AMINOACID SEQUENCES OF ISOFORM I(A) AND 2 (B) OF CETP

```
A       10         20         30         40         50         60
MLAATVLTLA LLGNAHACSK GTSHEAGIVC RITKPALLVL NHETAKVIQT AFQRASYPDI
        70         80         90        100        110        120
TGEKAMMLLG QVKYGLHNIQ ISHLSIASSQ VELVEAKSID VSIQNVSVVF KGTLKYGYTT
       130        140        150        160        170        180
AWWLGIDQSI DFEIDSAIDL QINTQLTCDS GRVRTDAPDC YLSFHKLLLH LQGEREPGWI
       190        200        210        220        230        240
KQLFTNFISF TLKLVLKGQI CKEINVISNI MADFVQTRAA SILSDGDIGV DISLTGDPVI
```

```
       250        260        270        280        290        300
TASYLESHHK GHFIYKNVSE DLPLPTFSPT LLGDSRMLYF WFSERVFHSL AKVAFQDGRL
       310        320        330        340        350        360
MLSLMGDEFK AVLETWGFNT NQEIFQEVVG GFPSQAQVTV HCLKMPKISC QNKGVVVNSS
       370        380        390        400        410        420
VMVKFLFPRP DQQHSVAYTF EEDIVTTVQA SYSKKKLFLS LLDFQITPKT VSNLTESSSE
       430        440        450        460        470        480
SVQSFLQSMI TAVGIPEVMS RLEVVFTALM NSKGVSLFDI INPEIITRDG FLLLQMDFGF
       490
PEHLLVDFLQ SLS
```

```
B       10         20         30         40         50         60
MLAATVLTLA LLGNAHACSK GTSHEAGIVC RITKPALLVL NHETAKVIQT AFQRASYPDI
        70         80         90        100        110        120
TGEKAMMLLG QVKYGLHNIQ ISHLSIASSQ VELVEAKSID VSIQNVSVVF KGTLKYGYTT
       130        140        150        160        170        180
AWWLGIDQSI DFEIDSAIDL QINTQLTCDS GRVRTDAPDC YLSFHKLLLH LQGEREPGWI
       190        200        210        220        230        240
KQLFTNFISF TLKLVLKGQI CKEINVISNI MADFVQTRAA SILSDGDIGV DISLTGDPVI
       250        260        270        280        290        300
TASYLESHHK AVLETWGFNT NQEIFQEVVG GFPSQAQVTV HCLKMPKISC QNKGVVVNSS
       310        320        330        340        350        360
VMVKFLFPRP DQQHSVAYTF EEDIVTTVQA SYSKKKLFLS LLDFQITPKT VSNLTESSSE
       370        380        390        400        410        420
SVQSFLQSMI TAVGIPEVMS RLEVVFTALM NSKGVSLFDI INPEIITRDG FLLLQMDFGF
       430
PEHLLVDFLQ SLS
```

MECHANISM FOR NEUTRAL-LIPID TRANSFER

The structure of the concave surface indicates that CETP can bind only one lipoprotein at a time. This provides strong support for the proposal that CETP operates by a carrier mechanism, in which CETP accepts neutral lipids from a donor particle, shuttles them through the aqueous phase and delivers them to an acceptor lipoprotein. In the more physiologically relevant hetero-exchange [123], CETP filled with cholesteryl ester (Figure: 8, step 1) binds VLDL and releases the bound phospholipid. As VLDL is triglyceride rich, one or two triglycerides can enter the tunnel and deposit an equal amount of cholesteryl ester into VLDL. The triglyceride-bound CETP departs from VLDL carrying two phospholipids from

the surface and travels through the aqueous plasma (step 2). It then engages HDL and releases the bound phospholipid (step 3). As HDL is cholesteryl ester rich, one or two new cholesteryl esters can enter the tunnel and an equal amount of bound triglyceride is deposited into HDL. The cholesteryl ester–filled CETP departs from HDL carrying two phospholipids from the surface (step 4) and hence completes a full cycle of hetero-exchange. The nonspecific nature of the tunnel suggests similar binding affinities for cholesteryl ester and triglyceride, consistent with CETP-mediated transfers being determined by the relative lipid compositions in lipoproteins.

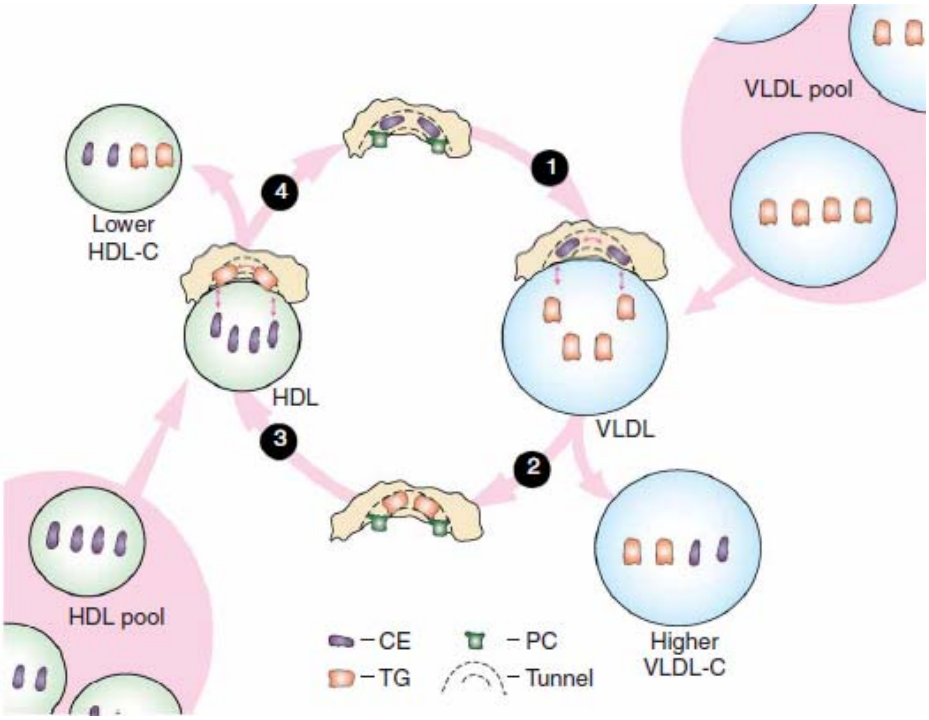


Figure 8: Proposed mechanism for CETP-mediated heteroexchange. VLDL (large circles) is normally triglyceride (TG) rich and HDL (smaller circles) is cholesteryl ester (CE) rich. Step 1, CETP filled with CE (as in the crystal structure) binds VLDL and releases the bound phospholipid (phosphatidylcholine, PC). One or two TGs enter the tunnel and an equal amount of CE is deposited into VLDL. Step 2, the TG-bound CETP dissociates from VLDL carrying two phospholipids from the surface, leaving the VLDL particle with a higher CE content. Step 3, the TG-bound CETP engages HDL and releases the bound phospholipid. One

or two new CEs enter the tunnel and an equal amount of TG is deposited into HDL. Step 4, the CE-filled CETP dissociates from HDL carrying two phospholipids from the surface and completes a full cycle of heteroexchange, which results in a lower CE content in HDL.

ROLE OF CETP IN PLASMA LIPID TRANSPORT AND IN ATHEROSCLEROSIS

The cardioprotective role of HDL is commonly explained by its function in the reverse cholesterol transport pathway, whereby excess cholesterol in peripheral tissues or atherosclerotic plaque is transported from vascular tissue back to the liver for metabolism and excretion in the bile.

Cholesteryl esters can be delivered directly to the liver by the interaction of HDL particles with the hepatic SR-B1 receptor or indirectly following the cholesteryl ester transfer protein (CETP) process.

Cholesteryl ester transfer protein plays an important role in reverse cholesterol transport. It transfers cholesteryl esters from HDL to LDL and VLDL particles in exchange for triglyceride, thereby reducing the concentration of HDL cholesterol and increasing non-HDL cholesterol, a lipoprotein distribution predisposing to atheroma formation. The cholesteryl ester in VLDL and LDL particles can then be taken up by all cells (both in liver and peripheral tissues) that express the LDL receptor (LDLr). Modified (oxidized) LDL is also taken up by macrophages in a scavenger receptor-mediated process that converts the macrophage into a foam cell. Accumulation of these foam cells in the arterial wall causes a chronic inflammatory reaction which leads to atherosclerosis. (Figure: 9)

The relationship of CETP to atherosclerosis was studied in a group of 28 cynomolgus monkeys fed high fat, high cholesterol diets for 5 years [150]. In these animals plasma CETP concentration showed a strong inverse correlation with HDL cholesterol concentration, and a

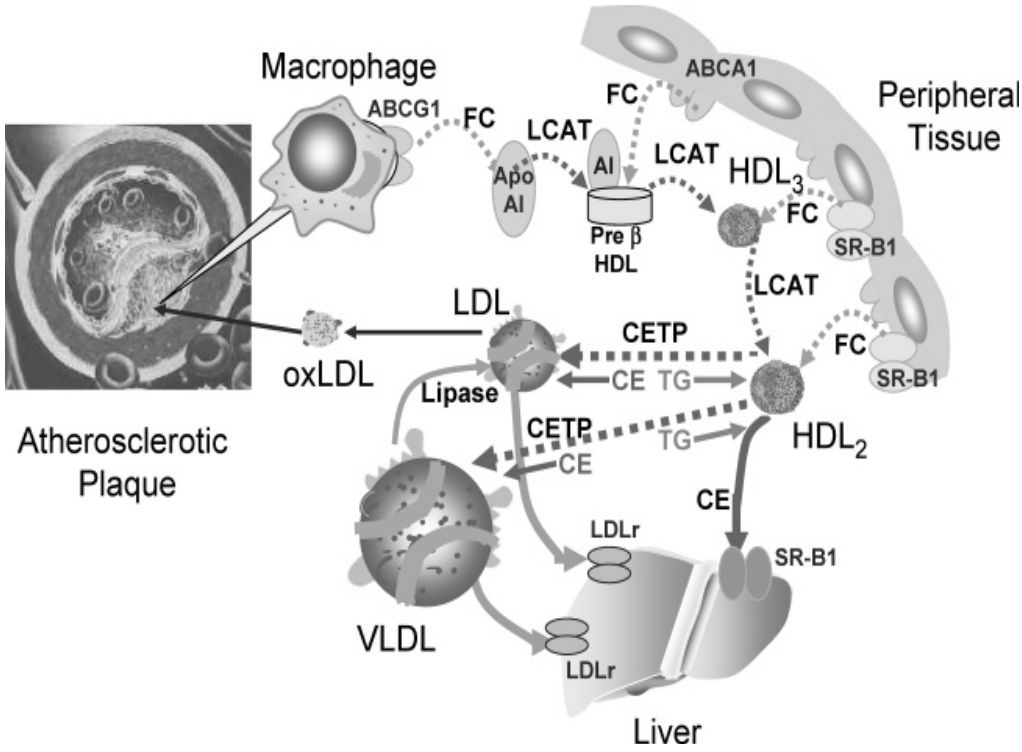
positive correlation with LDL cholesterol concentration and with LDL molecular weight. The extent of coronary artery atherosclerosis was positively correlated with LDL cholesterol concentration and with plasma CETP concentration. In multiple regression analysis only LDL cholesterol concentration appeared as an independent variable significantly correlated with coronary artery atherosclerosis. Thus, the positive correlation of CETP with atherosclerosis appeared to be due to its relationship with LDL cholesterol concentration. In cynomolgus monkeys the plasma CETP concentration appears to be a major determinant of the atherogenicity of the plasma lipoproteins, probably because CETP transfers from non-atherogenic or anti-atherogenic HDL to atherogenic LDL (or its precursors), and also because CETP may influence LDL particle number.

In many human dyslipidemias associated with accelerated atherosclerosis, there is an increase in plasma CETP concentration and/or an increase in the rate of net transfer of cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins in incubated plasma. These include dysbetalipoproteinemia[124], familial hypercholesterolemia[121], nephrotic syndrome[125], hypercholesterolemia[126,] insulin dependent diabetes mellitus [127], and peripheral vascular disease [128.]

Alcoholism, typically associated with increased HDL and decreased atherosclerosis, is associated with diminished CETP concentration [129]. Exercise conditioning, associated with increases in HDL and decreases in LDL cholesterol, is associated with decreased CETP concentration [130.]

The introduction of CETP into rats by injection or into mice by transgenesis [116] results in lipoprotein changes normally associated with the development of atherosclerosis.

FIG 9: ROLE OF CETP IN PLASMA LIPID TRANSPORT AND IN ATHEROSCLEROSIS



REGULATION OF CETP ACTIVITY OR EXPRESSION BY DIFFERENT FACTORS

The induction of hepatic CETP mRNA and plasma CETP by an atherogenic diet has been documented in rabbits, in cynomolgus and African Green monkeys, and in transgenic mice expressing the human CETP gene with its natural flanking sequences [131]. Detailed analysis of dietary variables shows that it is the cholesterol component of the diet which is largely responsible for the increase in CETP mRNA and protein. Exercise conditioning is associated with decreased plasma CETP concentration in human subjects, which may reflect both decreased adiposity and more effective insulin action. Plasma CETP concentration is inversely related to parameters of effective insulin action [132]. These findings suggest that

CETP mRNA in adipose and muscle could be decreased during feeding as a result of insulin action. The increase in CETP mRNA during fasting could be linked to the mobilization of fatty acids from the periphery, resulting in lipoprotein synthesis in the liver.

Plasma CETP is also altered by steroid hormone and drug stimuli. Plasma CETP activity is decreased as plasma cholesterol declines during pregnancy in the rabbit; however, plasma CETP is increased during the third trimester of human pregnancy, suggesting an effect of sex hormones on CETP expression. Corticosteroid therapy is associated with a reduction of plasma CETP in normal subjects and in patients with nephrotic syndrome [125].

Probucol therapy is associated with pronounced increases in plasma CETP [133.] Unexpectedly, probucol lowered CETP mRNA in human adipose tissue. The effect of probucol on CETP mRNA in cells may be conditioned by the cell's cholesterol pools.

Only limited information is available on the regulation of CETP expression in cultured cells, owing to the very low expression of CETP mRNA and protein in transformed cell lines. Several lines of evidence indicate that a variety of cell types respond to an increase in cholesterol load by increasing CETP secretion. Martin et al.[134] have determined the effects of apolipoprotein E genotype on the plasma lipoprotein and CETP responses to cholesterol feeding in young normal male subjects under rigorously controlled dietary conditions. ApoE genotype has significant and opposite effects on plasma CETP and HDL-C responses to dietary cholesterol in humans. One explanation for the effect of apolipoprotein E genotype on CETP levels is that CETP might be catabolized in association with remnant particles.

Lipolysis of VLDL by lipoprotein lipase stimulates the CETP-mediated transfer of cholesteryl ester from HDL to VLDL. This phenomenon is related to accumulation of fatty acids in the surface of VLDL, which greatly enhances the binding of CETP to these particles.

Also, lipolysis increases the binding of CETP to HDL, as a result of enrichment of HDL with phospholipids and fatty acids. HDL isolated from postprandial plasma is phospholipid enriched and acts as a more efficient substrate for CETP than fasting HDL [135]. A similar stimulation of transfer can be produced by the action of hepatic lipase or by the direct addition of fatty acids to the lipoprotein mixture. Barter and colleagues [136] have shown that the addition of fatty acids to a mixture of VLDL and HDL enhances unidirectional net transfer of cholesteryl ester from HDL to VLDL, without reciprocal transfer of triglycerides. This promotes the formation of very small HDL particles, probably equivalent to the pre-beta HDL, thought to be involved in the initial step of cholesterol efflux from cells into HDL**.** The ensemble of information suggests a physiological role for the stimulation of CETP-mediated lipid transfer by products of lipolysis.

Accelerated cholesteryl ester transfer between lipoproteins has been shown in Insulin dependent diabetics, perhaps reflecting accumulation of fatty acids in lipoproteins. The enrichment of lipoproteins with un-esterified cholesterol may also result in enhanced net cholesteryl ester transfer from HDL to VLDL. In general, the concentration of neutral lipids in the phospholipid surface of substrate particles appears to be an important determinant of lipid transfer activity. CETP-mediated lipid transfer between emulsions and LDL is enhanced by the presence of apolipoproteins, such as apolipoprotein A-I[137.] There do not appear to be specific protein co-factors influencing CETP activity. However, it has recently been shown that apolipoprotein A-II inhibits the production of smaller HDL by CETP [138].

Also, Kinoshita et al.[139] have recently shown, by apolipoprotein E supplementation of the plasma of an apolipoprotein E-deficient subject, that apolipoprotein E enhances lipid transfer from HDL to VLDL by enhancing the affinity of apolipoprotein E for VLDL.

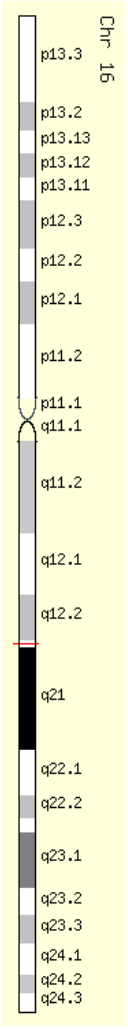
CETP GENE POLYMORPHISMS

CETP GENE

Human CETP gene is located in chromosome 16 (16q12-16q21) near the Lecithin-cholesterol acyltransferase (LCAT) gene locus. (Figure 10). It spans over 25 kb, consisting of 16 exons and 15 introns. The size of the exons ranges from 32 to 250 bp. Human CETP gene has a marked similarity to the plasma phospholipid transfer protein (PLTP) gene in the exon-intron organization [140]. Human PLTP transfers phospholipids between lipoproteins and has a 20% sequence homology to human CETP.

In the promoter region of the human CETP gene, sequences resembling a `TATA' box and an SP1-binding site were identified upstream from the translation start codon. The promoter region of human CETP gene contains a sequence resembling the binding site for the transcription factor CCAAT/enhancer-binding protein (C/EBP), which may be preserving CETP gene promoter activity [141]. ARP-1 (Apolipoprotein regulatory protein-***1)*** was shown to play a dichotomous role as both a transcriptional repressor and a transcriptional activator depending on the promoter context.

Fig 10: CHOLESTERYL ESTER TRANSFER PROTEIN GENE LOCUS – Chr 16 q21



CETP GENE POLYMORPHISMS AND CETP ACTIVITY

Several common restriction fragment length polymorphisms (RFLPs) have been reported in the human CETP gene, including TaqIA (intron 10), TaqIB (intron 1), StuI (exon 12) , MspI (intron 8) and EcoNI (intron 9)[22.]

TaqIB polymorphism (rs708272) is created by a silent base change affecting the 277th nucleotide in the first intron of the CETP gene[22], and possesses restriction site for the endonuclease TaqI, resulting in two alleles B1 and B2. Significant associations of the B1B1 genotype with higher plasma CETP concentration and/or CETP activity and lower HDL-c levels were found in several studies.[24-28] This association has been implicated to be population specific[142], and highly influenced by environmental factors such as alcohol consumption and tobacco smoking [143].

Isoleucine 405 to valine polymorphism (I405V) in exon 14 was also identified. This is a very common polymorphism, as the less-common allele is present in all populations examined and generally occurs at a frequency of over 25%. In multiple studies, those with the less-common 405V allele have lower CETP levels.[144]

Recently, a new functional polymorphism, CETP -629A/C has been identified.[145.] The -629A allele was associated with lower CETP mass and higher HDL cholesterol levels than the -629C allele. It was demonstrated that nuclear factors Sp1 and/or Sp3 suppress CETP promoter activity, whereas the binding of nuclear factors to the -629C allele has no effect on promoter expression. This is a unique polymorphism in the CETP gene promoter that may affect the promoter activity through the impaired binding of nuclear factors.

A one-nucleotide substitution (G to A) in exon 15, which changes arginine (451) to glutamine, was also identified.[146] Men heterozygous for the R451Q mutation showed 27% higher CETP activity than controls with normal genotype.

Regarding the gene defects,[147] a G-to-A mutation in the 5□-splice donor site of intron 14 was first identified in a Japanese patient with CETP deficiency. This defect was later identified in other Japanese CETP-deficient subjects and is associated with hyperalphalipoproteinemia (HALP). Human CETP deficiency is associated with both a decreased rate of production of apoB and an increase in its rate of catabolism, consistent with an up-regulation of the LDL receptor in these subjects.[148] Glueck et al. described families with familial HALP with hypobetalipoproteinemia, which was accompanied by longevity due to a low incidence of coronary heart disease [149].

A missense mutation (442D: G) in exon 15 was later identified. Although the patients were heterozygous for this defect, they had a 3-fold increase in plasma HDL cholesterol and markedly decreased plasma CETP activity and mass. A nonsense CETP mutation in exon 10 which causes a premature stop codon and another nonsense mutation in exon 6 (G181X) were also identified. The latter mutation causes a total deficiency of plasma CETP activity. Furthermore, a defect in the intron 10 splice donor site of CETP gene was found, which causes exon 10 skipping, resulting in abnormal downstream splice site selection. In the CETP-deficient patient with a heterozygous nonsense mutation (^{57}Tyr→stop), the presence of a null allele in addition to the allele with the nonsense mutation was suggested. This patient was accompanied by a marked postprandial lipemia. Figure 11 summarizes the site of gene mutations and polymorphisms in the CETP gene reported so far.

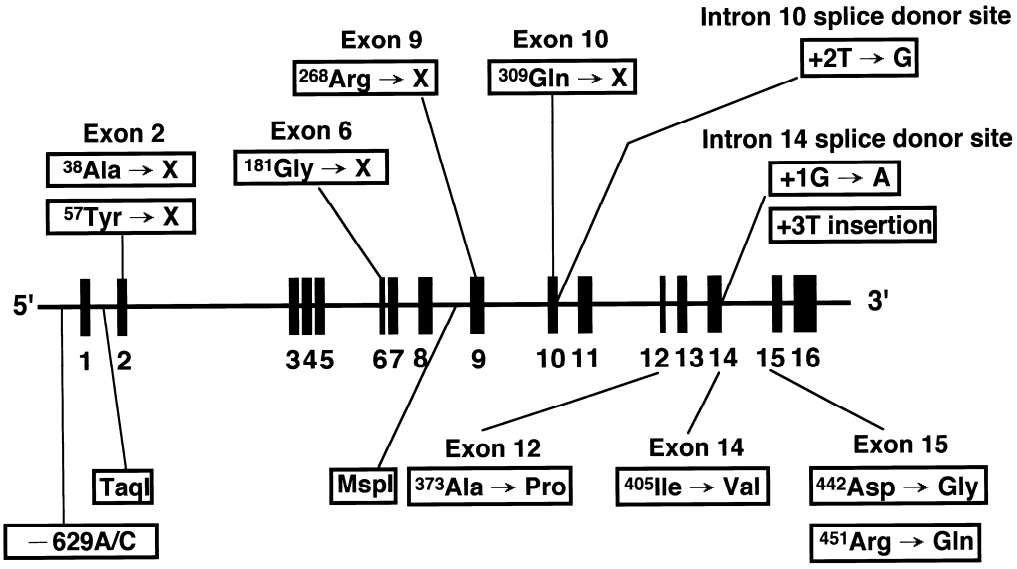


FIGURE 11 : POLYMORPHISMS AND MUTATIONS IN HUMAN CETP GENE

EFFECT OF CETP TaqIB POLYMORPHISM ON CETP ACTIVITY

The Taq1B polymorphism is very frequent, with the heterozygous B1B2 state being the most common. The common B1 (presence of the TaqI restriction site at this polymorphic site) allele generally is associated with increase in CETP activity and decreased HDL level when compared to B2 allele. Due to its intronic location this polymorphism cannot be considered as a part of a functional regulatory site, but can be a marker for another functional site. Taq1B polymorphism has been found to be in strong linkage disequilibrium with the -629C/A promoter mutation that occurs in a transcription factor binding site and affects Sp1/Sp3 binding [150]. There is also a promoter VNTR located 1,946 bp upstream of the transcription start site that is linked to Taq1B and appears to have an independent effect on CETP and HDL levels[151,152,153]. Other promoter polymorphisms like −971G/A and −1337C/T

have also been studied in combination with Taq1B and may also exert independent effects [32,33].

Subjects with genetic CETP deficiency have both elevated HDL cholesterol concentrations and decreased LDL cholesterol levels. A decrease in the catabolic rate of HDL apolipoproteins has also been reported. Although some of these effects are mediated through the actions of circulating CETP, CETP may also have an indirect effect on HDL and LDL by changing other potential lipoprotein receptors or apolipoprotein expressions. This view was supported by some studies indicating that CETP regulates the expression and secretion of an LDL receptor (LDL-R) and apolipoprotein A-I (apoA-I).

Several species, including mice and rats, are naturally deficient in CETP. Genetically engineered mice have proven to be valid models for the study of CETP function and its relation with atherosclerosis. Introduction of the human *CETP* gene into mice results in a dose-related reduction in HDL levels and a small increase in VLDL and LDL cholesterol and apolipoprotein B levels.[131,154] . As a consequence, these animals have significantly more early atherosclerotic lesions in the proximal aorta than do control mice. Mice are relatively resistant to the development of diet-induced atherosclerosis and must clearly accomplish reverse cholesterol transport by pathways that do not involve CETP activity. In fact, in studies of bile salt and cholesterol-fed, C57-B16 mice, the introduction and expression of the simian CETP gene resulted in enhanced formation of fatty streak lesions compared with non-expressing controls[116.] It was concluded that the enhancement of lesion development by CETP was secondary to a re-distribution of cholesterol from HDL to the VLDL/LDL fraction. These observations indicate the pro-atherogenic activities of CETP.

OTHER DISEASES ASSOCIATED WITH CETP GENE POLYMORPHISMS

As discussed above, a wide variety of cell types have been reported to secrete cholesteryl ester transfer activity, or protein, or to contain the CETP mRNA .Appreciable levels of cholesteryl ester transfer activity are present in seminal fluid and in cerebrospinal fluid. These findings strongly suggest that CETP influences lipid transfer processes in plasma, interstitial fluids, and within tissues and may play an important role in the development of various diseases such as atherosclerosis, diabetes mellitus, cholelithiasis, gall bladder cancer, age related macular degeneration, alzheimer's dementia and metabolic syndrome. Several laboratories have reported investigations into the relationship between the CETP gene polymorphisms and diseases other than CHD. Durlach et al. showed that French male type 2 diabetics are at increased risk of coronary heart disease with the B1 allele, while there was no effect in females [155].

CETP polymorphisms have been associated with increased risk for cholelithiasis. Juvonen et al. found that Finns with gall bladder disease have a higher risk of gallstones with the B1 allele when compared to those with B2 allele [156]. Sergio et al showed that on Chilean women the CETP TaqIB polymorphism was significantly associated with an increased risk of gall bladder cancer[157]. Lopez-Rios et al showed that the B1B1 genotype of the Taq1B *CETP* polymorphism is associated with more insulin resistance, higher post-oral glucose tolerance test glucose levels and an increased risk of Type 2 diabetes mellitus in Canarian population.[158.] Ukkola et al. have reported that the B1B1 genotype of this mutation was associated with cerebrovascular disease in Finnish patients with type 2 diabetes mellitus [159]. They also showed that individuals with the B2B2 genotype had a lower prevalence of signs of macroangiopathy. Meguro et al. found that Japanese type 2 diabetics have a higher likelihood of macroangiopathy with the B1B1 genotype than with the other genotypes [160.]

Radeau et al. showed that renal transplant recipients with the B1B1genotype were more likely to suffer CHD [161].

Cholesteryl ester transfer protein gene is involved in central nervous system cholesterol homeostasis and has been associated with exceptional longevity[162]. The ***CETP*** gene was identified as a "longevity gene" in a sample of Ashkenazi Jews by Barzilai and colleagues[162.]. They showed that individuals with exceptional longevity and their offspring were significantly associated with larger HDL and LDL particle sizes and CETP I405V polymorphism.

Sanders et al. showed that *CETP* I405V polymorphism is associated with slower memory decline and lower incident dementia and Alzheimer's dementia (AD) risk.[163.] The single population-based case-control study reported that the I405V polymorphism was associated with AD risk in non-***apolipoprotein E*** ε4 carriers.[164] A Spanish study reported that ε4 carriers homozygous for the minor allele of the ***CETP*** -629C/A polymorphism had a lower risk for AD than ε4 non-carriers but found no association between I405V and AD.[165] Alejandro et al. also suggested that the VV genotype of the I405V polymorphism of the CETP gene increases the risk of AD in the absence of the APOE4 allele [166.]

Cecile et al. found a significant association between the I405Vpolymorphism in the *CETP* gene with multiple features of the metabolic syndrome, i.e. the prevalence of abdominal obesity and prevalence of low HDL-cholesterol. For this polymorphism, the association with abdominal obesity was partly independent of the association with HDL-cholesterol, and vice versa.[167.] Anton et al. found that the rare B2 allele in the *CETP* gene significantly reduces the risk of the metabolic syndrome in subjects at high individual risk population [168.]

Chen et al found that rs3764261 polymorphism of CETP gene was associated with increased risk of age related macular degeneration. Because alleles near CETP gene are associated with decreased HDL levels in blood seem to increase the risk of age related macular degeneration.[169]

Additionally one study demonstrated that CETP TaqIB polymorphism was associated with atrial fibrillation. Asselbergs et al. fond that CETP TaqIB polymorphism is significantly associated with the presence of AF in the context of micro- or macroalbuminuria, elevated C-reactive protein, renal dysfunction, and ischemic heart disease. The underlying biological mechanism explaining the association between AF and the CETP TaqIB polymorphism remains unclear. A logical explanation would be that the widely acknowledged association between CETP gene variations (resulting in lower plasma CETP) and higher HDL cholesterol levels may prevent the initiation of (preclinical) atherosclerosis and subsequently the development of atrial fibrillation. This mechanism is very likely considering the modulating effect of ischemic heart disease on the association between CETP TaqIB polymorphism and AF in the present study.[170]

These studies provide an indication that CETP gene polymorphisms may play a wider role in the lipid transport, and that its role may not be restricted to atherosclerosis. As to the precise relationship between CETP gene polymorphisms and other diseases, as far as the current research condition is concerned, there is still a long way to go before we can come to a conclusion, and much further research is still needed.

AIM OF THE STUDY

AIM OF THE STUDY

Disturbances in lipoprotein metabolism play a major role in atherogenesis, a disease characterized by difference in susceptibility among people in any given population. A strong inverse relation exists between plasma high-density lipoprotein level and the risk of coronary artery disease. The cardio-protective role of HDL is commonly explained by its function in the reverse cholesterol transport pathway.

Cholesteryl ester transfer protein, a hydrophobic glycoprotein plays an important role in HDL metabolism and in reverse cholesterol transport pathway. Available reports address on the variability of cholesteryl ester transfer protein activity among people in any given population. This variability is attributed to the various polymorphisms of cholesteryl ester transfer protein gene.

In this scenario, this in-depth study of coronary heart disease was performed with the current strategy of investigating the contribution of genes to coronary heart disease in the general population and is based on the hypothesis that some genetic loci for proteins that influence the susceptibility of an individual to coronary heart disease are polymorphic and that the products of different alleles at a given locus have different effects on the disease. If an allele at a polymorphic site in or near a candidate gene (gene whose product is suspected to be involved in the pathogenesis of atherosclerosis and development of coronary heart disease) can be shown to be associated with coronary heart disease in unrelated people, this would indicate that the allele is causally related to the development of coronary heart disease.

The candidate gene of this study is Cholesteryl ester transfer protein gene and the aim of the study is,

- To determine the association of Cholesteryl ester transfer protein gene TaqIB polymorphism and the concerned Phenotype variation with Coronary artery disease.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY POPULATION

CASES

The study sample comprised 146 unrelated Coronary Artery Disease patients (131 male, 15 female) of Mean age 50.82 + 9.27 years. Inclusion criteria was more than 50% stenosis of at least one of the major coronary arteries. Patients with less obstruction were excluded. Patients with recent episode of Myocardial infarction (Less than 3 months) were also excluded.

CONTROL SUBJECTS

Controls were recruited from out-patient department during their visit for non- cardiac cases. Age, Sex and other confounding factors like diabetes, hypertension, smoking, alcoholism were matched. For all diabetic controls, tread mill test was done. Only those with negative Tread Mill Test result were included in the study.

METHODS

Recumbent blood pressure and 12 lead ECG were recorded on each subject after a thirty minute rest on the couch. Height and weight were recorded and blood samples were collected by Venipuncture after fortnight fasting in two test tubes. One was collected into plain nonadditive tube and the other anticoagulated with EDTA. Plain tube was centrifuged at 2000 rpm for 20 minutes and serum was utilised for lipid profile estimation. EDTA-containing tube was centrifuged at 2000 rpm for 20 minutes to get the buffy coat for DNA extraction and plasma was used for estimation of CETP activity.

BUFFY COAT SEPARATION

Buffy coat was separated by centrifugation of EDTA tubes at 2000 revolutions per minute for 20 minutes. Buffy coat was transferred to 2mL eppendorf and was used for DNA extraction. Plasma separated was used for estimation of CETP activity.

BIOCHEMICAL MARKERS

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglyceride concentration (TGL) were determined enzymatically using kits and XL-300 auto analyzer at Centralized Biochemistry Laboratory at G.G.H, Chennai-3.

DNA EXTRACTION BY MODIFIED HIGH SALT METHOD[171]

RBC Lysis:

- 400µL of buffy coat in a 2mL eppendorf is mixed with 1.6mL of 0.17M ammonium chloride and mixed by inversion until red cells are lysed for about 10 minutes
- The cells are centrifuged at 4000rpm for 10minutes.
- The white cell pellet is washed with 800µL of 0.17M ammonium chloride solution. The procedure is repeated till a clear white cell pellet is obtained.

WBC Lysis

- To the pellet 500 µL of TKM I solution is added. It is centrifuged at 10,000rpm for 10minutes.

Nuclear Lysis

- Discard the supernatant. To the pellet add 500 µL of TKM II solution. To that add 300 µL of 6M Nacl and 50 µL of 10% SDS.
- Mix well (vortex), Centrifuge at 10,000 rpm for 10 minutes.
- Save the supernatant. Transfer it to 1.5mL eppendorf.

DNA Precipitation

- To the supernatant double the volume of 100% ethanol is added.
- The sample is stored at -20◦C for 1 hour.
- Then it is centrifuged at 10,000 rpm for 20minutes at 4◦C in a refrigerated centrifuge.
- The supernatant is discarded. To this 500 µL of 70% ethanol is added. The pellet is mixed and centrifuged at 10,000 rpm for 10minutes at 4◦C.
- Supernatant is discarded and the pellet is air dried.

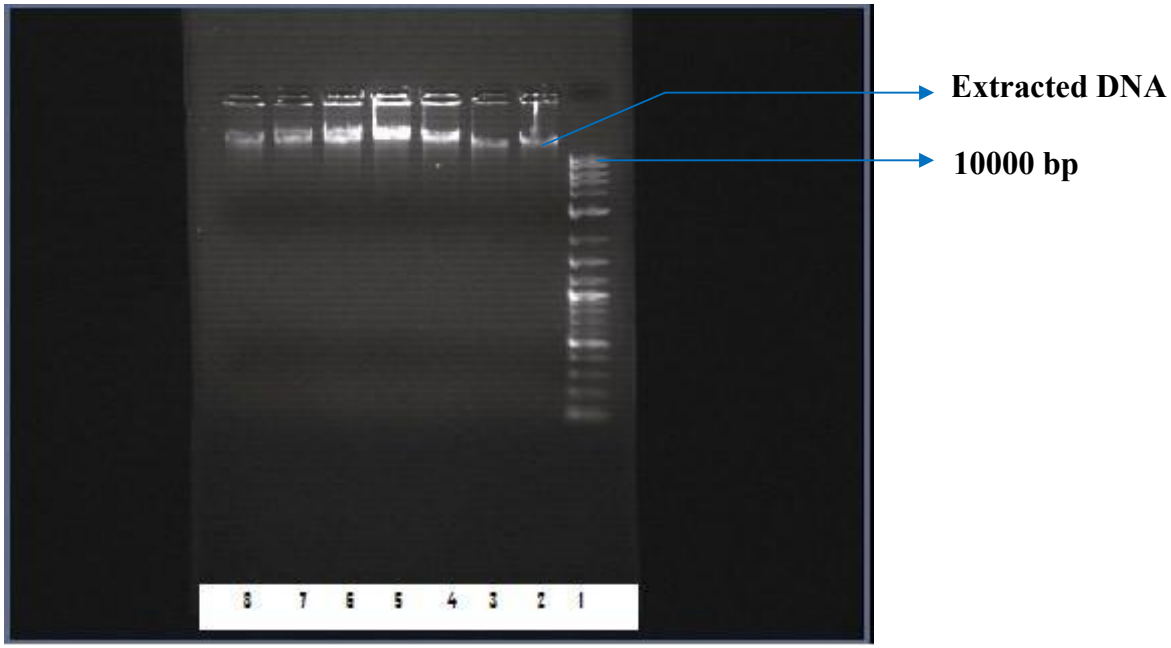
Storage

- To the pellet 30 µL of LTE buffer is added and the extracted DNA is stored at -20◦C for future use.

Identification

- Extracted DNA was identified by 0.8% agarose gel electrophoresis with a constant voltage of 7V/ cm and comparison with a known molecular weight 1kb DNA ladder.Figure:12.

DNA EXTRACTION BY HIGH SALT METHOD



- **Figure :12 shows, extracted DNA (lane 2 to 8) was tested on 0.8% agarose gel using 1kbp ladder (lane 1)**

- **Ladder shows 10000, 8000, 7000,6000, 5000, 4000, 3000, 2000 and 1000 bp fragments**

Concentration of extracted DNA

- Concentration of extracted DNA was estimated using UV spectroscopy at 260nm.The absorbance at 260nm was 0.0204. Concentration was calculated using the formula: 1 OD is equivalent to 50µg/mL

Conc. of DNA = absorbance × 50µg/mL × dilution factor

= 0.0204 × 50 × 100

= 102 ng / µL

- Purity of extracted DNA was assessed by 260/280 ratio and it was found to be > 1.7

POLYMERASE CHAIN REACTION

- 535 bp fragment of CETP gene was amplified using,
    - Forward primer – 5'-CACTAGCCC AGAGAGAGGAGTGCC–3'
    - Reverse primer - 5'-CTGAGCCCAGCCGCACACTAAC–3'

Primer Reconstitution

Primers are supplied in lyophilized form. Autoclaved distilled water is used to prepare 100 × concentrations i.e. 10times the molecular weight of primer is the volume of water required to prepare 100 × concentrations which is 100µmolar solution.

- From this stock solution 10 × concentration is prepared as the working solution for PCR.

MASTER MIX:

- Genei Red Dye master mix in the following composition was used.

- Master Mix consists of a unique inert red dye in addition to basic components necessary for PCR.

    - Reaction buffer consisted of Tris Hcl - 10mM at pH 8.3

      KCl - 50mM

    - MgCl2 - 1.5mM acts as catalyst.
    - dNTP's were used in a concentration of 2.5mM each.
    - Taq polymerase in a concentration of 1.5 U.

- Primers were used in a concentration of 9 pmol and DNA was used in a concentration of 200ng.

- PCR was carried out in a reaction volume of 25 µL with the following components;

    - PCR master mix – 12.5 µL
    - Forward primer – 0.9 µL
    - Reverse primer – 0.9 µL
    - DNA – 2 .0µL
    - Distilled water – 8.7 µL
    - Total – 25 µL

- Amplification was carried out in an Applied Biosystems thermal cycler with the following cycling conditions.

Initial denaturation – 95^0 C -5min

- 30 cycles of
    - Denaturation – 95^0C – 1 min
    - Annealing - 65^0C – 1min
    - Extension - 72^0C – 1min
    - Final extension at 72^0C - 10 min.

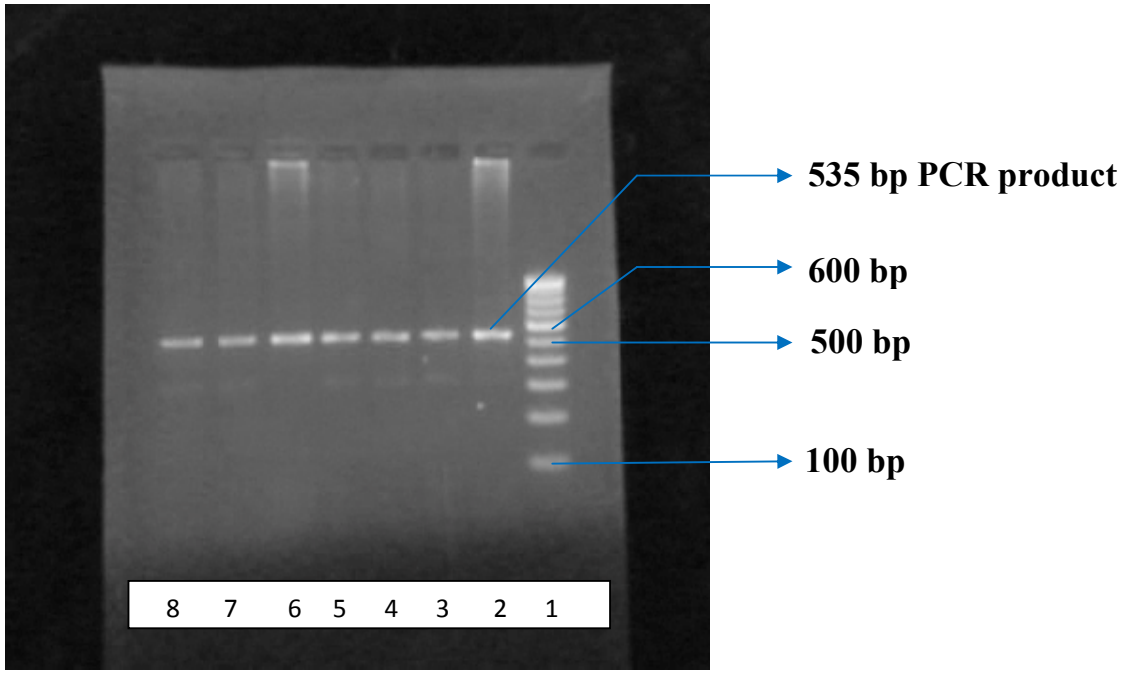
- Amplified product – amplicons of 535 bp was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder. Figure : 13.

AGAROSE GEL ELECTROPHORESIS

- PCR product is run on 2% agarose gel in a 30 mL agarose cast as follows: 0.6g of agarose is weighed and dissolved in 30mL of TAE buffer with a pH of 8.0.

- It is microwaved for 60 secs, cooled and 1.5 µL of ethidium bromide (10mg/mL) is added. It is poured into a cast and allowed to solidify for 15 min before it is kept in the electrophoresis tank. 8 µL of PCR product is loaded onto wells and 4 µL of 100bp DNA ladder is loaded onto single well as a marker. It is electrophoresed at 8V/cm for 45min and visualized under UV illumination.

POLYMERASE CHAIN REACTION



- **Figure shows the 535bp CETP gene PCR product (lane 2 to 8) on 2% agarose gel.**
- **Lane1 shows 100bp DNA ladder – marker fragments include 1000, 900, 800,700, 600, 500, 400, 300, 200, 100bp.**

RESTRICTION DIGESTION OF PCR PRODUCTS

CETP TaqIB polymorphism was detected by digestion of the PCR amplified product with the TaqI restriction enzyme (4 units for 2 hours) followed by size fractionation in 2% agarose gel electrophoresis.

Principle of Taq1 enzyme digestion

- B2 allele does not have the restriction site hence will yield a 535bp fragment.
- B1 allele has the restriction site, hence gets cleaved to give 361bp and 174bp fragment.
- Heterozygous individuals (B1B2) have 535bp, 361bp and 174bp fragments.
- Analysis was done using a 100bp DNA ladder from Bangalore genei.

Procedure

- 16 µL of PCR product is aliquoted in an eppendorf and 4U of TaqI enzyme is added. The entire procedure is carried out in ice. The contents are mixed thoroughly.
- The eppendorf is then placed in a 65^0C waterbath for 2 hours and reaction is stopped by adding 5 µL of gel loading dye and mixed thoroughly.
- Restriction digested product is subjected to 2% agarose gel electrophoresis for genotyping.

AGAROSE GEL ELECTROPHORESIS

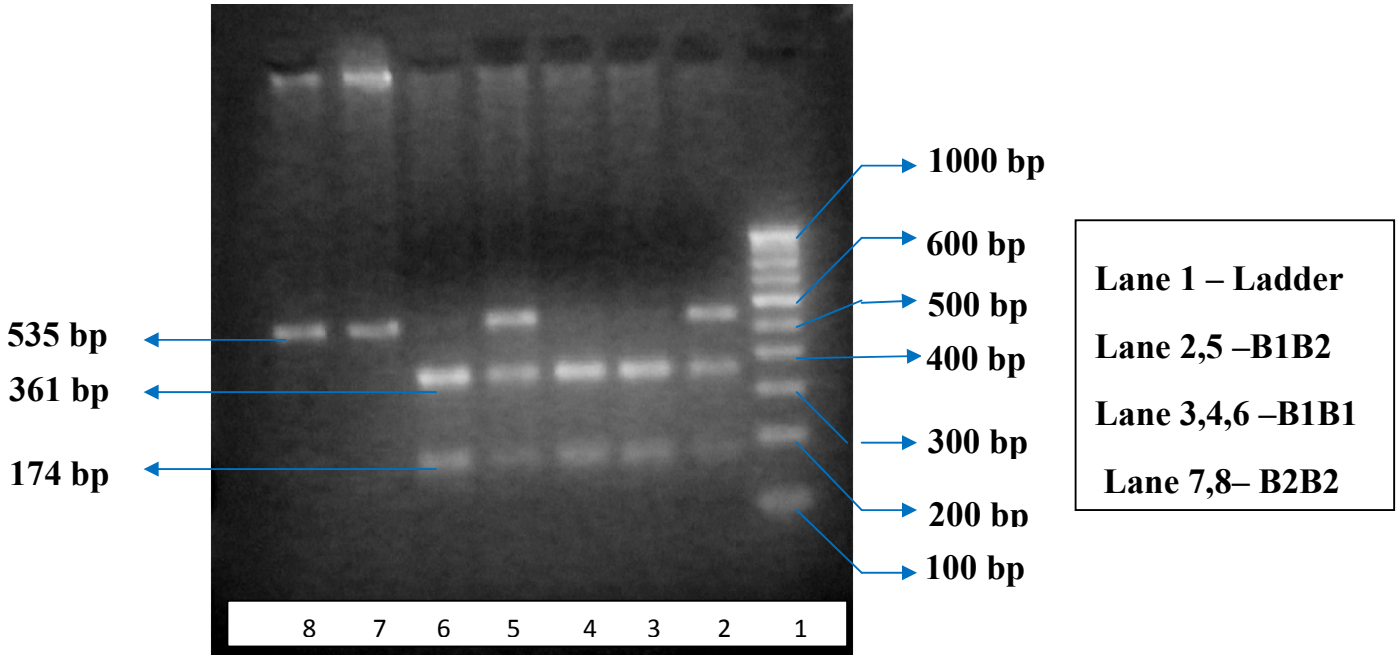
- 2% agarose gel electrophoresis is used for genotyping of CETP TaqIB polymorphism.

- Restriction digested PCR amplicons were electrophoresed on 2% agarose gel in a 60 mL agarose cast as follows: 1.2 g of agarose is weighed and dissolved in 60mL of TAE buffer with a pH of 8.0.

- It is microwaved for 60 secs, cooled and 3 µL of ethidium bromide (10mg/mL) is added. It is poured into a cast and allowed to solidify for 15 min before it is kept in the electrophoresis tank.

- 16 µL of PCR product is loaded onto wells and 4 µL of 100bp DNA ladder is loaded onto single well as a marker. It is electrophoresed at 8V/cm for 45min and visualized under UV illumination. Figure : 14.

RESTRICTION DIGESTION PRODUCTS



- **Figure shows genotype analysis done on 2% agarose gel electrophoresis using 100bp DNA ladder (lane 1).**

- **It shows the following fragments – 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100bp.**

LIPID PROFILE

The biochemical parameters undertaken for the study were determined using the following methodologies:

Estimation of Plasma Total Cholesterol

Method

Cholesterol Esterase – Cholesterol Oxidase

Kit used

Liquixx of ERBA diagnostics Mannheim GmbH Ltd.

Principle

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Quinoneimine), which is measured at 505nm.

Reagent composition:

Goods buffer (pH - 6.4) 100mmol/L

Cholesterol Esterase > 200U/L

Cholesterol Oxidase > 100U/L

Peroxidase > 3000 U/L

4- Aminoantipyrine 0.3 mmol/L

Phenol 5mmol/L

Non - reactive stabilizers & surfactants

Standard (Cholesterol 200mg/dL)

Cholesterol 2g/L

Procedure

To 1 mL of the reconstituted reagent, 10 µL of plasma is added and reading is taken after 5 mins of incubation at 37^{o} C.

Reference Values

Cholesterol : 150-260 mg /dL

Estimation of Plasma Triglyceride

Method

Enzymatic Colorimetric method

Kit Used

Liquixx of ERBA diagnostics Mannheim GmbH Ltd.

Principle

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Free Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol -3- Phosphate} + \text{ADP}$$

$$\text{Glycerol -3- Phosphate} + O_2 \xrightarrow{\text{Glycerol -3- Phosphate Oxidase}} \text{Dihydroxy acetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4 Aminoantipyrine} + \text{3,5-DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2H_2O$$

The intensity of chromogen (Quinoneimine) formed during the reaction is directly proportional to the triglyceride concentration in the sample and is measured at 505nm.

Reagent composition:

Pipes buffer (pH - 7.0)	40 mmol/L
Lipoprotein Lipase	4000 U/L
Glycerol Kinase	1500 U/L
Glycerol -3- Phosphate Oxidase	4000 U/L
Peroxidase	2200 U/L
4- Aminoantipyrine	0.4 mmol/L
ATP	2.0 mmol/L
Magnesium (Mg^{+2})	2.5 mmol/L
DHBS (3,5-Dichloro - 2 hydroxy benzene sulphonate)	0.2 mmol/L

Standard (Triglycerides 200mg / dL)

Glycerol (Trig. Equivalent) 2g / L

Procedure

To 1 mL of the reconstituted reagent 10 µL of plasma is added and read at 505 nm after incubation at 37^{o}C for 5minutes.

Reference Range

Males 60- 165 mg/dL

Females 40- 140 mg/dL

Estimation of HDL Cholesterol

Method

Modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method

Kit used

Erba XL System Packs

Principle

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and chylomicron (CM) by cholesterol oxidase (CHOD) and cholesterol esterase

(CHER). The enzymes selectively react with HDL to produce hydrogen peroxide which yields a blue coloured complex upon oxidase condensation with TODB (N, N - Bis (4-sulfobutyl)-3-methylaniline) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) . The intensity of chromogen (Quinone) formed during the reaction is directly proportional to the HDL-C concentration in the sample and is measured at 593 nm.

$$\text{HDL + LDL + VLDL +CM} \xrightarrow[\text{PEGME}]{\text{PVS}} \text{HDL + (LDL + VLDL +CM) . PVS/PEGME}$$

$$\text{HDL + } H_2O \text{ + O2} \xrightarrow{\text{CHER \& CHOD}} \text{Cholest-4-en-3-one + Fatty acid + } H_2O_2$$

$$2H_2O_2 \text{ + 4-AA + TODB} \xrightarrow{\text{Peroxidase}} \text{Quinone + 5 } H_2O$$

Reagent composition:

Reagent 1

MES buffer (pH - 6.5)	6.5 mmol
TODB (N,N-Bis(4-sulfobutyl)-3-methylaniline))	3 mmol
Polyvinyl sulfonic acid (PVS)	50 mg/L
Polyethylene-glycol-methyl ether	30 ml/L
Magnesium chloride ($MgCl_2$)	2 mmol
EDTA	
Detergent	

Reagent 2

MES buffer (pH - 6.5)	50 mmol
Cholesterol oxidase	20 kU/L

Cholesterol esterase	5 kU/L
Peroxidase	5 kU/L
4- Aminoantipyrine	0.9 g/L
Detergent	0.5%

Calibrator

HDL-C 60 mg/dL

Procedure

Reagent 1 & 2 are placed in the auto analyzer with the following assay parameters:

Assay type	: 2 point
Primary wavelength nm	: 600
Secondary wavelength nm	: 700
R-1 volume	: 210
R-2 volume	: 70
Reaction direction	: Increasing
Sample volume	: 3 µL
Calibration	: Straight

Reference Values

Adult male	: 35.3 – 79.5 mg /dL
Adult female	: 42.0 – 88.0 mg / dL

Estimation of LDL Cholesterol

Method

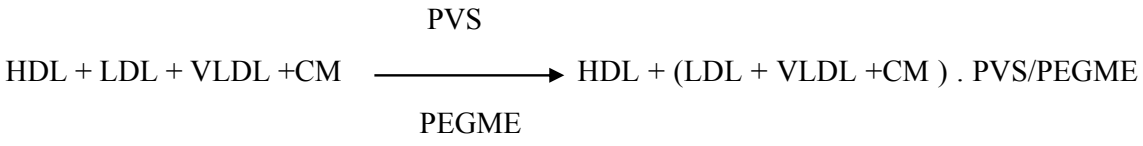
Modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method

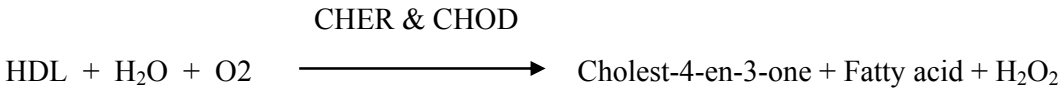
Kit used

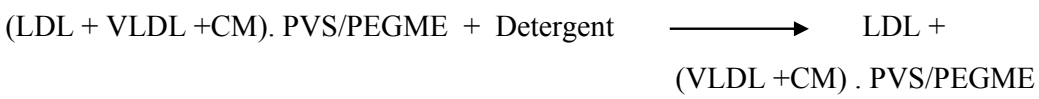
Erba XL System Packs

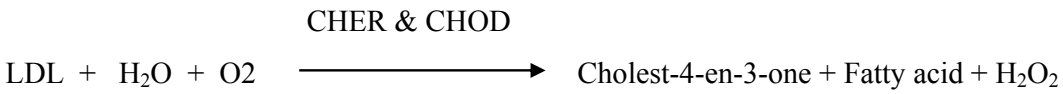
Principle

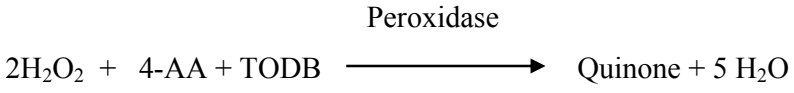
The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and chylomicron (CM) by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of reagent 2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce hydrogen peroxide which yields a blue colored complex upon oxidase condensation with TODB (N,N-Bis (4-sulfobutyl) -3-methylaniline) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) . The intensity of chromogen (Quinone) formed during the reaction is directly proportional to the HDL-C concentration in the sample and is measured at 593 nm.











Reagent composition:

Reagent 1

MES buffer (pH - 6.5)	50 mmol
Polyvinyl sulfonic acid (PVS)	50 mg/L
Polyethylene-glycol-methyl ether	30 ml/L
Magnesium chloride ($MgCl_2$)	2 mmol
EDTA	
Detergent	
Cholesterol oxidase	20 kU/L
Cholesterol esterase	5 kU/L
Peroxidase	5 kU/L

4- Aminoantipyrine	0.9 g/L

Reagent 2

MES buffer (pH - 6.5)	50 mmol
EDTA	
Detergent	
TODB (N,N-Bis(4-sulfobutyl)-3-methylaniline))	3 mmol

LDL Calibrator

LDL-C	107 mg/dL

Procedure

Reagent 1 & 2 are placed in the auto analyser with the following assay parameters:

Assay type	:	2 point
Primary wavelength (nm)	:	600
Secondary wavelength (nm)	:	700
Reagent-1 volume	:	210
Reagent-2 volume	:	70
Reaction direction	:	Increasing
Sample volume	:	3 µL
Calibration	:	Straight

Expected Values

Optimal	< 100 mg/dL
Near/above optimal	100 - 129 mg/dL
Boderline high	130 to 159 mg/dL
High	160 to 189 mg/dL
Very high	> 189 mg/dL

VLDL Cholesterol

VLDL-C was calculated by using the formula given below:

VLDL-C = TGL/5

Estimation of Cholesteryl ester transfer protein activity:

Cholesteryl ester transfer protein activity in plasma was measured by using Fluorometric assay kit.

Principle

The CETP Activity Assay Kit uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP[172.] CETP-mediated transfer of the fluorescent neutral lipid to the acceptor

molecule results in an increase in fluorescence which is read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm.

Kit Contents:

Components	Volume
Donor molecule	1 ml
Acceptor Molecule	1 ml
CETP Assay Buffer (10X)	5 ml
Positive Control (Rabbit Serum)	30 µL

Preparation of Standard Curve:

Standard curve is prepared by making serial dilutions of the donor molecule in isopropanol and subsequently recording the fluorescence intensity of each dilution, using isopropanol alone as a blank.

1. 6 test tubes are labeled T0 to T5, each contains 0.2 ml of isopropanol and the tube labeled T5 contains an additional 0.2 ml of isopropanol.

2. 2 µL of Donor Molecule is added to T5 and mixed well.

3. 0.2 ml is transferred from T5 to T4 and mixed well. Then 0.2 ml is transferred from T4 to T3 and mixed. Then 0.2 ml is transferred from T3 to T2 and mixed. Then 0.2 ml is transferred from T2 to T1. The Donor Molecule solution contains 0.1 mM labeled lipids and thus the standard curve samples contain 0, 6.25, 12.5, 25, 50, 100 pmol donor molecule.

4. The fluorescence intensity (Excitation = 465 nm; Emission = 535 nm) of the samples from T0 to T5 are read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm.

5. The fluorescence intensity values of the standard curve are applied directly to our results to express activity of the plasma sample (pmoles/μL plasma/hr).

Procedure

For each reaction - 10 μL donor molecule, 10 μL acceptor molecule, 20 μL 10X CETP assay buffer, 3 μL sample, distilled water to a total of 200 μL are added and incubated for 1 hour at 37°C. A blank is prepared that contains water instead of CETP source. The samples are diluted to 500 μL with 1X CETP assay buffer and read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm. A standard curve is used, according to the manufacturer's guidelines, to derive the relation between fluorescence intensity and mass transfer. The activity is expressed in pmol/μL/hr.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

1. Allele frequencies were calculated by allele counting.

2. Age, BMI, serum lipid levels were compared between control subjects and patients by Student's t tes .

3. Genotype frequency distribution between cases and controls were compared with a χ^2 test for 2*2 contingency table.

4. Plasma Cholesteryl ester transfer protein activity was compared between control and cases by Student's t test $p<0.05$ was considered significant.

5. Plasma Cholesteryl ester transfer protein Activity, serum HDL-C and serum LDL-C were compared between CETP Taq1B genotypes by using one-way ANOVA and Student's t test.

6. Logistic regression analysis was performed to evaluate the interaction between human CETP Taq1B genotypes and other variables in relation to the prevalence of Coronary Artery Disease. Independent variables included in the analysis were age (quantitative), sex (male/female), smoking (yes/no), Alcoholism (Yes/No), Hypertension (Yes/No), Diabetes (Yes/No), Serum Levels of Cholesterol , Triglycerides (Quantitative). The analysis was executed by SAS Statistical program Version 6.10 for Macintosh.

7. Plasma Cholesteryl ester transfer protein activity level, serum High Density Lipoprotein levels and serum Low Density Lipoprotein levels were correlated by Pearson's Correlation analysis.

8. Relationship between the number of Coronary arteries and the Plasma Cholesteryl ester transfer protein activity was assessed by Spearman's Rank Correlation analysis.

RESULTS

MASTER CHART

Table - 1 : LIPID LEVELS, BMI AND GENOTYPE OF CASES

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	WT (Kg)	HT (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
1	44	M	SVD	No	No	Yes	Yes	66	1.57	26.78	159	194.7	26.8	89.3	79.68	B1B2
2	41	M	TVD	No	Yes	Yes	No	71	1.64	26.48	175.3	180.5	44.8	90.4	94.47	B1B1
3	40	M	DVD	Yes	No	No	Yes	63	1.57	25.68	175.7	175.6	44.7	91.9	90.58	B1B1
4	57	M	SVD	No	No	Yes	No	68	1.62	25.89	167.9	116.9	50.1	90.4	78.94	B1B2
5	59	F	DVD	No	Yes	No	No	59	1.52	25.77	196.7	194.5	24.4	129.4	86.75	B1B1
6	45	M	TVD	Yes	No	Yes	No	78	1.68	27.67	197.6	197.8	23.5	130.5	114.57	B1B1
7	46	M	SVD	No	Yes	No	Yes	58	1.64	21.67	174.7	96.8	59.4	91.9	86.94	B1B2
8	49	M	SVD	No	Yes	No	No	72	1.71	24.78	188.5	185.7	22.8	124.6	99.2	B1B1
9	40	M	SVD	No	No	Yes	No	74	1.57	29.98	214	195.7	40.4	130.5	86.01	B1B2
10	48	M	DVD	No	Yes	Yes	Yes	81	1.59	31.99	161.8	180.8	44.1	77.5	104.29	B1B1
11	44	M	SVD	No	No	Yes	No	58	1.64	21.57	158.8	184.8	40.3	77.5	82.02	B1B2
12	62	M	SVD	No	Yes	No	Yes	64	1.7	21.99	215.5	193.6	38.3	134.5	91.73	B1B1
13	50	M	TVD	Yes	Yes	Yes	No	71	1.62	26.87	210.2	198.9	31.9	134.5	107.49	B1B1
14	51	M	DVD	No	Yes	Yes	No	61	1.7	21.24	146.2	88.8	39.5	84.9	64.4	B2B2
15	59	F	SVD	No	Yes	No	No	56	1.61	21.78	207.7	182.5	36.8	130.4	89.54	B1B2
16	48	M	TVD	Yes	Yes	No	Yes	80	1.58	32.01	203.7	196.9	29.9	130.4	99.58	B1B1

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
17	40	M	SVD	No	No	Yes	No	62	1.69	21.78	160.5	188.4	38.5	80.3	79.49	B1B2
18	40	M	SVD	No	Yes	No	No	71	1.54	29.87	150.4	98.5	46.4	80.3	102.64	B1B1
19	51	M	SVD	Yes	No	Yes	No	57	1.64	21.24	176.9	98.4	57.4	95.8	75.43	B1B2
20	59	M	SVD	No	Yes	No	No	59	1.72	20.01	209.8	189.4	37.4	130.5	61.81	B2B2
21	49	M	TVD	Yes	No	Yes	Yes	79	1.71	27.02	168.1	106.5	52.6	90.2	81.93	B1B2
22	48	M	SVD	No	Yes	Yes	Yes	54	1.53	22.87	168.7	188.4	36.7	90.3	86.02	B1B2
23	58	M	TVD	No	Yes	No	Yes	83	1.64	30.88	174.6	196.9	35.4	95.8	102.81	B1B1
24	56	M	SVD	Yes	No	Yes	No	60	1.63	22.66	176.6	189.5	34.2	100.5	89.93	B1B2
25	54	M	DVD	Yes	Yes	No	Yes	62	1.61	23.98	179.7	87.3	63.7	94.5	54.44	B2B2
26	59	F	SVD	No	Yes	No	No	72	1.71	24.56	160.3	197.8	26.5	90.2	106.69	B1B1
27	59	M	DVD	No	No	Yes	Yes	69	1.67	24.78	206.3	185.7	32.4	132.8	74.81	B1B2
28	43	M	SVD	No	Yes	Yes	No	61	1.6	23.67	216.4	90.2	64.9	129.5	53.45	B2B2
29	47	M	DVD	Yes	Yes	No	Yes	69	1.71	23.68	172.2	84.6	56.8	94.5	90.81	B1B2
30	41	M	SVD	No	Yes	Yes	No	70	1.65	25.77	170.3	196.4	34.6	92.4	92.82	B1B2
31	40	M	TVD	No	Yes	No	Yes	75	1.58	29.99	175.3	186.7	33.7	100.3	112.64	B1B1
32	56	M	DVD	Yes	Yes	No	Yes	73	1.62	27.68	162.2	193.5	36.7	82.8	83.64	B1B2
33	58	M	SVD	No	No	Yes	No	58	1.6	22.79	199.4	83.6	53.8	124.9	58.86	B2B2
34	52	M	DVD	No	Yes	Yes	No	61	1.58	24.57	184.8	188.4	36.8	106.3	78.63	B1B2
35	40	M	DVD	No	Yes	No	Yes	84	1.71	28.67	201.4	197.8	28.3	129.5	119.68	B1B1
36	50	M	TVD	No	No	Yes	Yes	76	1.75	24.67	175.9	84.8	59.2	95.7	85.49	B1B2

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
37	42	M	SVD	Yes	Yes	No	No	64	1.64	23.68	201.9	184.6	51.4	109.6	61.82	B2B2
38	38	M	SVD	No	Yes	Yes	Yes	55	1.6	21.55	213.9	179.6	39.3	134.7	100.2	B1B2
39	56	F	TVD	Yes	Yes	No	No	77	1.62	29.46	206.8	190.8	31.8	132.8	108.69	B1B1
40	55	M	SVD	No	No	Yes	Yes	60	1.57	24.47	205.2	195.9	24.6	137.4	79.69	B1B2
41	40	M	TVD	No	No	Yes	Yes	60	1.66	21.79	152.5	78.5	51.9	80.9	78.84	B1B2
42	39	M	DVD	Yes	Yes	No	Yes	67	1.61	25.89	166.5	189.7	30.1	94.5	106.75	B1B1
43	46	M	DVD	Yes	Yes	No	No	69	1.64	25.66	200.6	130.6	50.2	120.3	86.06	B1B2
44	62	M	TVD	No	Yes	Yes	Yes	71	1.63	26.89	178.8	188.9	34.6	102.4	92.98	B1B1
45	32	M	SVD	No	No	Yes	No	67	1.67	23.99	156.1	96.8	49.9	82.8	104.41	B1B1
46	50	M	TVD	Yes	Yes	No	Yes	68	1.59	26.79	211.2	135.8	37.9	142.1	91.63	B1B2
47	48	M	DVD	No	No	Yes	Yes	62	1.55	25.87	183.6	188.3	25.6	116.3	108.64	B1B1
48	57	M	DVD	No	No	Yes	No	60	1.56	24.57	249.4	184.6	54.7	153.8	75.93	B1B2
49	52	M	TVD	Yes	Yes	No	Yes	76	1.6	29.68	169.3	184.7	22.7	105.7	108.59	B1B1
50	59	M	TVD	No	Yes	No	Yes	66	1.67	23.55	194.6	186.9	46.3	106.9	86.32	B1B2
51	51	M	DVD	Yes	No	Yes	Yes	73	1.72	24.57	209.6	195.4	31.8	134.7	114.68	B1B1
52	49	M	SVD	No	No	Yes	Yes	57	1.63	21.46	211.2	193.7	52.7	115.8	61.91	B2B2
53	59	M	DVD	Yes	Yes	No	Yes	68	1.65	24.88	169.3	74.7	50.1	100.3	74.81	B1B2
54	48	M	TVD	No	No	Yes	Yes	74	1.67	26.43	208.1	185.7	29.6	137.4	97.59	B1B1
55	64	F	SVD	Yes	Yes	No	No	63	1.63	23.54	151.8	80.6	39.2	92.5	83.24	B1B2
56	50	M	DVD	No	No	Yes	Yes	79	1.69	27.71	179.2	180.5	38.9	100.2	85.39	B1B2

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
57	56	M	DVD	No	Yes	Yes	No	68	1.62	25.81	150.7	88.4	48.1	80.9	108.92	B1B1
58	61	M	SVD	Yes	No	Yes	No	62	1.66	22.54	168.9	187.8	43.1	84.2	63.81	B2B2
59	44	M	TVD	No	No	Yes	No	73	1.74	24.21	241.9	96.9	59.1	159.4	60.34	B1B2
60	41	M	TVD	Yes	No	No	Yes	81	1.64	29.98	191.5	185.3	30.1	120.3	104.69	B1B1
61	41	M	DVD	No	Yes	Yes	No	76	1.69	26.53	167.8	189.8	33.4	92.4	93.68	B1B2
62	62	M	SVD	No	No	Yes	No	73	1.72	24.72	208.7	154.7	39.9	133.9	64.68	B2B2
63	47	M	TVD	No	No	Yes	No	72	1.65	26.54	167.7	74.8	60.1	88.6	69.47	B1B2
64	42	M	SVD	Yes	No	Yes	No	78	1.71	26.84	217.2	191.2	32.9	142.1	99.19	B1B1
65	47	F	SVD	No	Yes	No	No	56	1.58	22.34	172.8	183.7	38.7	93.4	83.79	B1B2
66	54	M	TVD	No	No	Yes	Yes	74	1.67	26.65	161.7	96.3	49.1	89.3	116.4	B1B1
67	60	M	DVD	No	No	Yes	No	73	1.65	26.88	209.9	190.5	35.2	132.6	87.93	B1B2
68	59	M	TVD	No	Yes	Yes	No	68	1.57	27.74	222.2	199.2	24.6	153.8	108.89	B1B1
69	53	M	DVD	No	No	No	Yes	73	1.57	29.71	171.6	190.7	34.9	94.6	86.91	B1B2
70	43	M	TVD	No	No	Yes	No	72	1.67	25.82	183.4	193.2	23.9	116.9	114.9	B1B1
71	42	M	TVD	No	No	Yes	Yes	80	1.64	29.81	209.3	175.4	40.1	130.1	69.02	B1B2
72	34	M	SVD	Yes	Yes	No	No	57	1.68	20.24	208.5	180.6	38.7	129.7	69.91	B2B2
73	39	M	TVD	No	No	Yes	Yes	80	1.68	28.53	185.3	198.3	31.3	110.3	102.69	B1B1
74	40	M	SVD	No	No	Yes	No	73	1.66	26.41	189.3	165.6	58.7	93.5	69.49	B1B2
75	56	M	SVD	No	Yes	No	No	57	1.63	21.58	169.42	164.6	39.7	92.8	71.92	B2B2
76	57	M	DVD	Yes	No	Yes	Yes	78	1.65	28.75	253.2	189.4	56.5	154.8	79.69	B1B2

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
77	59	F	TVD	No	Yes	No	No	75	1.64	28.02	185.9	189.7	31.5	112.5	98.26	B1B1
78	64	M	SVD	No	No	Yes	No	64	1.65	23.53	136.3	184.8	40.5	54.8	64.57	B2B2
79	36	M	TVD	Yes	No	No	Yes	69	1.67	24.65	143	74.7	34.8	89.3	78.91	B1B2
80	49	M	DVD	No	No	Yes	Yes	82	1.66	29.83	212	194.6	36.4	132.7	64.91	B2B2
81	68	M	DVD	No	No	Yes	Yes	74	1.6	28.93	219.9	190.7	34.2	143.6	95.68	B1B2
82	57	F	TVD	Yes	Yes	No	No	73	1.62	27.72	156.9	89.6	34.8	100.2	115.49	B1B1
83	62	M	DVD	No	No	Yes	No	78	1.62	29.83	168.7	190.4	37.2	89.4	84.64	B1B2
84	51	M	TVD	No	Yes	No	No	76	1.65	27.81	231.7	184.3	31.4	159.4	109.68	B1B1
85	65	M	DVD	No	No	Yes	Yes	74	1.64	27.51	202.6	190.5	34.6	125.9	95.83	B1B2
86	53	M	SVD	No	No	Yes	No	73	1.71	24.92	123.5	83.9	39.8	62.9	70.1	B2B2
87	59	M	SVD	Yes	No	No	No	57	1.66	20.61	169.4	196.8	35.7	90.3	94.39	B1B2
88	56	M	DVD	No	No	Yes	Yes	75	1.64	27.9	138.4	196.9	38.7	56.3	69.54	B2B2
89	68	F	DVD	Yes	Yes	No	No	80	1.65	29.51	164.2	177.5	36.8	87.9	89.65	B1B2
90	59	M	SVD	No	No	No	Yes	55	1.63	20.71	212.7	154	42.1	135.8	65.84	B2B2
91	39	M	DVD	No	No	Yes	Yes	73	1.63	27.61	226.3	197.6	36.9	145.9	93.01	B1B2
92	53	M	TVD	No	Yes	Yes	No	78	1.62	29.84	167.1	189.7	32.8	92.4	104.69	B1B1
93	63	M	SVD	Yes	No	Yes	No	52	1.55	21.51	160.5	151.4	35.7	90.5	89.76	B1B2
94	66	M	TVD	No	Yes	Yes	Yes	57	1.54	23.96	169.7	192.4	38.6	88.6	110.6	B1B1
95	63	M	SVD	No	No	Yes	No	62	1.65	22.65	151.7	198.9	43.2	64.7	68.53	B2B2

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
96	54	M	DVD	Yes	No	No	Yes	82	1.66	29.72	153.9	172.5	34.9	80.49	94.92	B1B2
97	63	M	TVD	Yes	No	No	No	69	1.64	25.83	192.2	175.3	29.7	123.4	122.89	B1B1
98	59	M	SVD	No	Yes	Yes	No	66	1.69	22.94	160.6	164.6	38.9	84.8	94.71	B1B2
99	50	M	TVD	No	No	Yes	No	70	1.62	26.83	200.2	196.4	24.3	132.6	108.89	B1B1
100	66	M	TVD	Yes	Yes	No	No	82	1.66	29.83	206.9	111.9	35.7	144.8	89.76	B1B2
101	38	M	DVD	No	Yes	Yes	No	79	1.62	29.97	154.5	141.6	34.6	87.6	98.78	B1B2
102	61	M	SVD	Yes	No	Yes	No	79	1.61	30.64	157.8	189.8	21.2	94.6	110.68	B1B1
103	49	M	TVD	No	No	Yes	Yes	65	1.66	23.46	191.6	175.4	22.4	130.1	101.61	B1B1
104	36	M	SVD	No	No	Yes	Yes	58	1.64	21.42	193.3	193.5	35.8	114.8	94.97	B1B2
105	67	M	TVD	Yes	No	No	Yes	69	1.67	24.67	186.9	164.7	36.5	113.5	110.67	B1B1
106	56	M	DVD	Yes	No	Yes	No	76	1.6	29.65	153.8	76.9	55.1	79.3	68.97	B1B2
107	67	F	TVD	Yes	Yes	No	No	66	1.66	23.86	198.5	85.6	22.6	154.8	105.4	B1B1
108	60	M	DVD	Yes	No	No	Yes	64	1.58	25.74	162.5	193.5	26.1	93.7	96.03	B1B2
109	51	M	DVD	No	Yes	Yes	No	63	1.59	24.87	162.1	152.4	27.2	100.4	96.04	B1B2
110	67	M	TVD	Yes	No	Yes	Yes	70	1.68	24.76	158.4	154.7	34.2	89.3	110.53	B1B1
111	57	M	TVD	No	No	No	Yes	74	1.55	30.75	180.6	183.5	35.3	104.6	93.92	B1B2
112	53	M	SVD	Yes	No	Yes	No	61	1.62	23.41	186.6	126.4	36.9	120.4	94.96	B1B2
113	41	M	TVD	No	Yes	No	Yes	64	1.65	23.53	221.3	197.8	34.1	143.6	98.97	B1B1
114	44	M	SVD	Yes	No	Yes	No	53	1.62	20.31	160	162.4	32.6	91.9	93.76	B1B2

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
115	59	M	SVD	No	No	Yes	No	61	1.69	21.42	208.1	185.8	36.4	130.5	94.02	B1B2
116	43	M	TVD	No	No	Yes	Yes	80	1.64	29.56	158.9	88.8	47.7	89.4	110.09	B1B1
117	50	M	TVD	No	Yes	No	No	75	1.61	28.75	196.9	195.7	27.9	125.9	109.97	B1B1
118	56	F	DVD	No	Yes	No	No	79	1.62	29.97	139.7	189.4	40.3	57.5	86.03	B1B2
119	41	M	SVD	No	No	Yes	No	49	1.56	20.22	179.2	169.3	36.8	104.54	94.96	B1B2
120	53	M	TVD	No	Yes	No	Yes	70	1.61	26.98	162.5	183.8	31.4	90.3	110.48	B1B1
121	46	M	DVD	Yes	No	Yes	No	80	1.63	29.94	116.7	90.4	44.2	50.4	64.73	B2B2
122	34	M	SVD	No	No	Yes	No	58	1.59	22.86	168.2	184.6	26.9	100.4	97.92	B1B2
123	67	F	TVD	No	Yes	No	No	82	1.64	30.46	203	197.1	31.7	127.9	118.58	B1B1
124	58	F	DVD	Yes	No	No	No	79	1.6	30.76	168.1	179.7	27.9	100.3	97.95	B1B2
125	34	M	SVD	No	No	Yes	Yes	53	1.61	20.55	151.5	93.4	41.9	86.9	56.46	B2B2
126	64	M	SVD	No	Yes	Yes	No	67	1.65	24.75	119.3	91.5	41.2	55.8	84.83	B1B2
127	34	M	TVD	No	No	Yes	Yes	82	1.66	29.75	221.9	187.9	34.4	145.9	98.48	B1B1
128	54	M	DVD	Yes	No	Yes	Yes	80	1.64	29.64	183.5	192.4	40.8	100.2	85.64	B1B2
129	40	M	SVD	No	No	Yes	No	63	1.59	24.88	120.1	103.6	37.1	58.3	89.54	B1B2
130	49	M	TVD	No	No	Yes	Yes	77	1.6	29.98	189.9	196.4	46.1	100.5	84.64	B1B1
131	39	M	TVD	No	No	Yes	Yes	63	1.64	23.35	155	84.8	54.5	80.49	90.57	B1B1
132	64	F	SVD	Yes	No	No	No	62	1.71	21.08	209.7	190.3	37.2	130.4	87.96	B1B2
133	48	M	TVD	No	No	No	Yes	75	1.57	30.56	151.8	88.5	45.3	84.8	110.57	B1B1

Sl. No	Age (yr)	Sex	Angio Finding	DM	HYT	SMK	ALC	Wt. (Kg)	Ht. (m)	BMI (kg/m^2)	CHOL (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	CETP Activity (pmol/µL/hr)	CETP TaqIB Genotype
134	45	M	TVD	No	No	Yes	Yes	66	1.67	23.56	148.3	83.7	51.8	75.8	75.74	B1B2
135	39	M	SVD	No	No	Yes	No	70	1.71	23.98	218.4	197.6	30.1	144.8	89.91	B1B1
136	64	M	SVD	Yes	Yes	No	No	69	1.68	24.42	138.2	147.9	40.1	64.5	86.86	B1B2
137	66	M	TVD	No	No	Yes	Yes	76	1.57	30.94	173.4	88.4	54.1	97.6	109.59	B1B1
138	39	M	DVD	Yes	No	No	No	79	1.66	28.57	188.2	192.5	37.9	107.8	94.83	B1B2
139	41	M	SVD	No	Yes	No	No	55	1.63	20.87	222.2	192.6	36.9	142.8	96.64	B1B2
140	42	M	TVD	No	No	Yes	Yes	82	1.65	29.96	193.1	193.5	35.6	114.8	91.89	B1B1
141	41	M	SVD	No	No	No	Yes	82	1.69	28.75	167.5	180.6	40.5	86.9	83.03	B1B2
142	35	F	DVD	Yes	Yes	No	No	70	1.56	28.85	160.4	83.5	50.4	89.3	104.89	B1B1
143	48	M	SVD	Yes	No	No	No	66	1.54	27.65	157.3	195.8	38.4	75.7	96.94	B1B2
144	52	M	TVD	No	No	Yes	Yes	76	1.65	27.84	164.2	197.8	26.9	93.7	109.99	B1B1
145	46	M	SVD	No	Yes	No	No	68	1.65	24.86	146.4	186.4	40.4	64.7	86.75	B1B2
146	41	M	DVD	No	No	Yes	Yes	74	1.66	26.97	188.5	193.6	25.4	120.4	110.83	B1B1

TABLE 3: CHARACTERISTICS OF PATIENTS WITH CAD AND OF CONTROL SUBJECTS

Variables	Case	Control	P value
Age	50.82 ± 9.3	50.81 ± 8.8	0.99 –NS
Sex male	131 (89.7%)	128 (88.3%)	0.69 –NS
Female	15 (10.3%)	17 (11.7%)	
DM	48 (51.1%)	46 (48.9%)	0.83 –NS
HT	61 (48.8%)	64 (51.2%)	0.69 –NS
DM+HT	23 (15.8%)	20 (13.8%)	0.61 – NS
SMK	87 (54%)	74 (46%)	0.14 –NS
ALC	66 (52.8%)	59 (47.2%)	0.44 –NS
BMI	25.36 ±3.25	24.99 ± 3.14	0.27 –NS
Total cholesterol	180.9 ±26.7	159.9 ±23.2	0.000 –S
Triglycerides	161.6 ±42.8	128 ±27.6	0.000 –S
High Density lipoprotein	38.5 ±9.7	48.2±9.9	0.000 –S
Low Density lipoprotein	106 ±25.3	82.1±25.1	0.000-S

TABLE 4: GENOTYPE DISTRIBUTION OF HUMAN CETP GENE

Genotype	Control	Case	P value
B1B1	31 (21.4%)	56 (38.4%)	Chi sq = 19.18 p= 0.000 – S
B1B2	67 (46.2%)	71 (48.6%)	
B2B2	47 (32.4%)	19 (13%)	

FIGURE 15: GENOTYPE DISTRIBUTION OF HUMAN CETP GENE

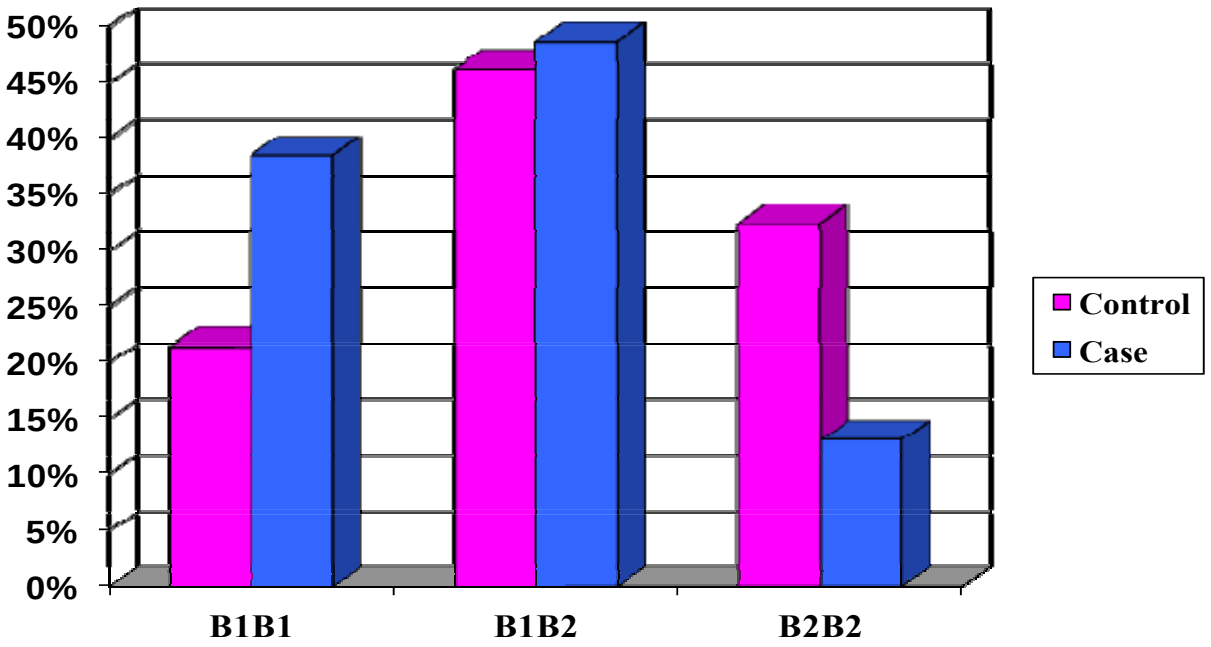


TABLE 5: COMPARISON OF CETP ACTIVITY AMONG CONTROLS AND CASE

Variable	Control	Case	P value
CETP ACTIVITY (pmol/μL/hr)	65.23 ± 2.23	90.72±15.83	0.000 –S

FIGURE 16: COMPARISON OF CETP ACTIVITY AMONG CONTROLS AND CASES

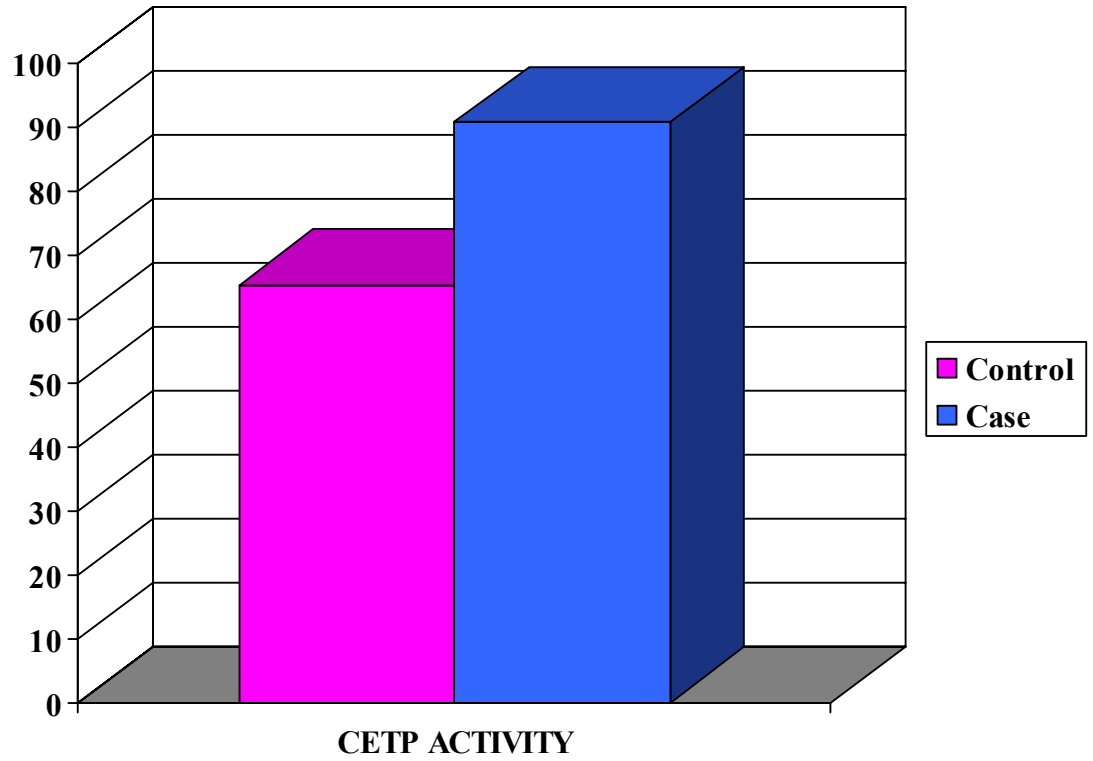


TABLE 6: RELATIONSHIP BETWEEN CETP ACTIVITY AND GENOTYPES

Variable	B1B1 Genotype	B1B2 Genotype	B2B2 Genotype	P value
CETP Activity (pmol/μL/hr)	97.09	74.53	59.1	0.000-S

FIGURE 17 : RELATIONSHIP BETWEEN CETP ACTIVITY AND GENOTYPES

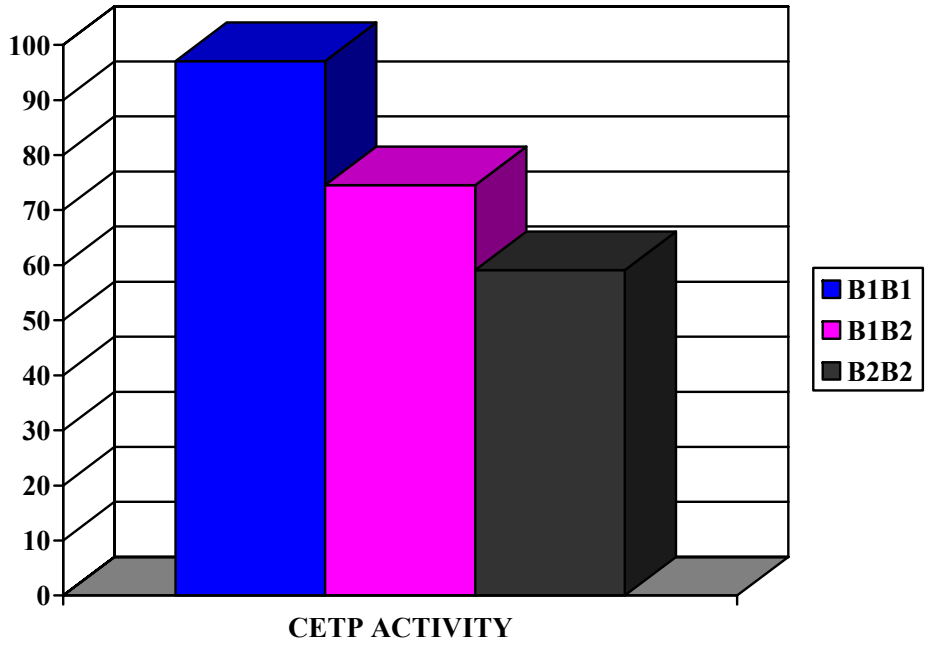


TABLE 7: RELATIONSHIP BETWEEN HDL-C, LDL-C AND GENOTYPES

Variable	B1B1	B1B2	B2B2	P Value
HDL-C	37	44.6	50.2	0.0002
LDL-C	104.9	91.9	82.3	0.0004

FIGURE 18 : RELATIONSHIP BETWEEN HDL-C, LDL-C AND GENOTYPES

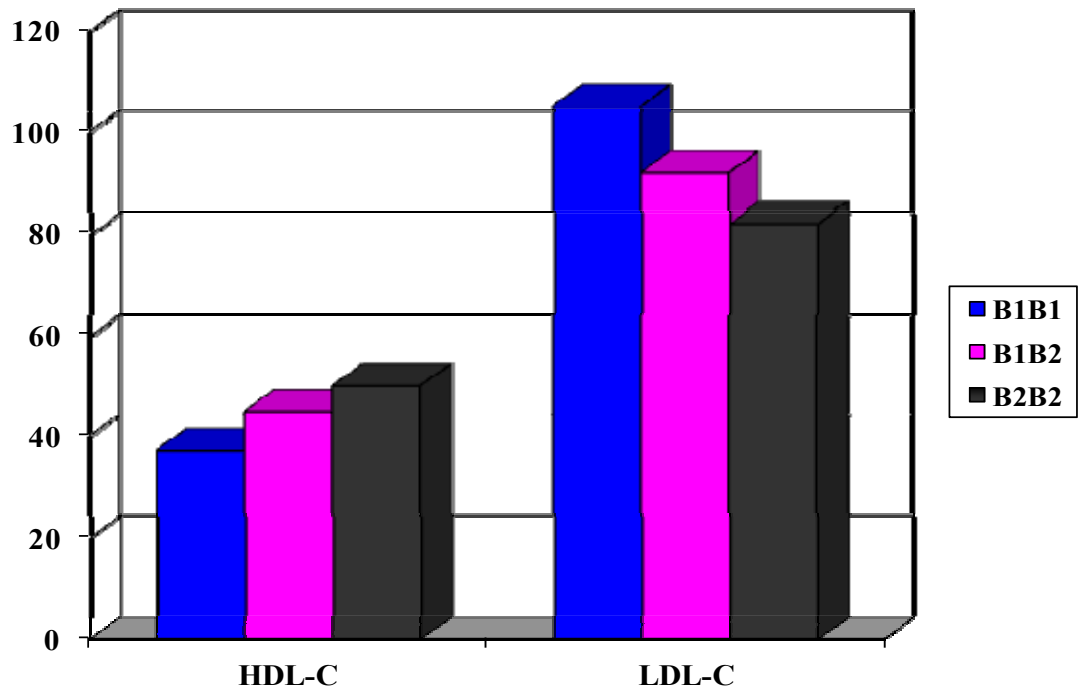


TABLE 8 : CORRELATION BETWEEN HIGH DENSITY LIPOPROTEIN AND CETP ACTIVITY

Group			HDL	CETP ACTIVITY
Control	HDL	Pearson correlation Sig. (2-tailed) N	1 145	-.716** .000 145
	CETP ACTIVITY	Pearson correlation Sig. (2-tailed) N	-.716** .000 145	1 145
Case	HDL	Pearson correlation Sig. (2-tailed) N	1 146	-.544** .000 146
	CETP ACTIVITY	Pearson correlation Sig. (2-tailed) N	-.544** .000 146	1 146

**Correlation is significant at the 0.01 level (2-tailed).

TABLE 9 : CORRELATION BETWEEN LOW DENSITY LIPOPROTEIN AND CETP ACTIVITY

Group			LDL	CETP ACTIVITY
Control	LDL	Pearson correlation	1	.443**
		Sig. (2-tailed)		.000
		N	145	145
	CETP ACTIVITY	Pearson correlation	.443**	1
		Sig. (2-tailed)	.000	
		N	145	145
Case	LDL	Pearson correlation	1	.204**
		Sig. (2-tailed)		.014
		N	146	146
	CETP ACTIVITY	Pearson correlation	.204**	1
		Sig. (2-tailed)	.014	
		N	146	146

**Correlation is significant at the 0.01 level (2-tailed).

TABLE 10: CORRELATION BETWEEN CETP ACTIVITY AND NUMBER OF VESSELS STENOSED

			CETP Activity (pmol/µL/1hr)	Angio Finding
Kendall's tau_b	CETP Activity (pmol/µL/1hr)	Correlation Coefficient	1.000	.325(**)
		Sig. (2-tailed)	.	.000
		N	291	146
	Angio Finding	Correlation Coefficient	.325(**)	1.000
		Sig. (2-tailed)	.000	.
		N	146	146
Spearman's rho	CETP Activity (pmol/µL/1hr)	Correlation Coefficient	1.000	.417(**)
		Sig. (2-tailed)	.	.000

	N	291	146
Angio Finding	Correlation Coefficient	.417(**)	1.000
	Sig. (2-tailed)	.000	.
	N	146	146

** Correlation is significant at the 0.01 level (2- tailed).

TABLE 11: UNIVARIATE ANALYSIS

GENOTYPE	CONTROLS (n)	CASES (n)	ODDS RATIO	P VALUE
B1B1	31	56	3.1 (1.8 - 5.4)	0.000
B1B2	67	71	2.3 (1.3 – 3.8)	0.002
B2B2	47	19	1.0	

TABLE 12: MULTIVARIATE LOGISTIC REGRESSION ANALYSIS

VARIABLES	B	S.E.	WALD	Sig.	Exp (B)	95% CI	
						UPPER	LOWER
AGE	0.017	0.022	0.619	0.432	1.017	0.975	1.061
SEX	-0.664	0.746	0.791	0.374	0.515	0.119	2.222
SMOKING	0.814	0.558	2.126	0.145	2.257	0.756	6.739
ALCOHOLISM	-0.188	0.443	0.179	0.672	0.829	0.348	1.977
DIABETES	0.422	0.666	0.400	0.527	1.525	0.413	5.628
HYPERTENSION	-0.092	0.592	0.024	0.877	0.912	0.286	2.909
DIABETES+ HYPERTENSION	0.254	0.876	0.084	0.772	1.289	0.232	7.174
BMI	-0.028	0.062	0.207	0.649	0.972	0.862	1.097
TOATL HOLESTEROL	0.025	0.008	7.811	0.003	1.025	1.009	1.042
TRIGLYCERIDES	0.011	0.006	3.297	0.069	1.011	0.999	1.023
HDL	-0.072	0.028	6.604	0.010	1.075	1.017	1.136
LDL	0.237	0.557	3.123	0.000	1.534	1.096	2.167
CETP ACTIVITY	0.197	0.726	4.505	0.000	1.218	1.156	1.283
CETP POLYMORPHISM	0.844	0.674	8.802	0.000	2.040	1.011	3.150
CONSTANT	-2.081	1.140	4.075	0.014	0.103		

RESULTS

a. Table 3 shows Age, Sex, BMI, High Density Lipoprotein levels, Low Density Lipoprotein levels and conventional risk factor distribution among patients and control subjects. We obtained a non-significant p value with respect to all the confounding variables like age, sex, BMI, history of diabetes, hypertension, smoking, alcoholism. There was a significant difference in the Total cholesterol level (high in cases - 180.9 +26.7, low in controls - 159.9 +23.2), Triglycerides level (high in cases - 161.6 +42.8, low in controls - 128 +27.6), Low Density lipoprotein level (high in cases - 106 +25.3, low in controls - 82.1+25.1) and High Density Lipoprotein level (low in cases - 38.5 +9.7, high in controls - 48.2+9.9).

b. Table 4 shows Genotype distribution of human CETP gene in patients with CAD and control subjects. The Allele frequencies were B1B1 = 87, B1B2 =138 and B2B2 = 66. This was found to be in Hardy Weinberg equilibrium.

- B1B1 genotype was more frequent among cases (38.4%) when compared to controls (21.4%). In contrast B2B2 was more common among controls (32.4%) when compared to cases (13%). There was a significant difference in the distribution of B1B2 genotype also between cases (48.6%) and controls (46.2%). P value is 0.000.

- Table 5 shows the comparison of CETP activity among cases and controls. Significantly high CETP activity could be observed among cases (90.72+ 15.83) when compared to controls (65.23 + 12.23).

- Table 6 shows the difference in CETP activity between B1B1 genotype, B1B2 genotype and B2B2 genotype. The activity was significantly higher among B1B1 genotype (97.09 pmol/μL/hr; p = 0.000 vs B2B2) and B1B2 genotype individuals (74.53 pmol/μL/hr; p = 0.000 vs B2B2) when compared to B2B2 genotype individuals (59.1).

- Table 7 shows the difference in HDL-C and LDL-C levels between B1B1 genotype, B1B2 genotype and B2B2 genotype. The HDL-C was significantly lower among B1B1 genotype (37 mg/dL; p value = 0.000 vs B2B2) and B1B2 genotype individuals (44.6 mg/dL; P = 0.001 vs B2B2) when compared to B2B2 genotype individuals (50.2 mg/dL). The LDL-C was significantly higher among B1B1 genotype (104.9 mg/dL; p value = 0.000 vs B2B2) and B1B2 genotype individuals (91.9 mg/dL; P = 0.05 vs B2B2) when compared to B2B2 genotype individuals (82.3 mg/dL).

- Table 8 shows the correlation between High Density Lipoprotein levels and CETP Activity. There was a strong negative correlation between High Density Lipoprotein levels and CETP activity among controls and cases.

- Table 9 shows the correlation between Low Density Lipoprotein levels and CETP Activity. There was a strong positive correlation between Low Density Lipoprotein levels and CETP activity among controls and cases.

- Table 10 shows the correlation between CETP activity and the number of vessels stenosed. Single vessel disease was ranked 1, double vessel disease 2 , Triple Vessel

disease 3 . There was a positive correlation between activity and the rank. i.e as the activity increased number of vessels affected increased.

- Table 11 shows the Odds ratio calculation on Univariate analysis to evaluate the risk of atherosclerosis among B1B1 genotype individuals (Odds ratio is 3.1; 95% CI – 1.8 to 5.4) and B1B2 genotype individuals (Odds ratio is 2.3; 95% CI – 1.3 to 3.8).

- On multivariate analysis (Table 12), we obtained a significant negative correlation coefficient for HDL levels as expected i.e., an increase in the HDL levels decreased the possible occurrence of atherosclerosis, whereas there was a positive correlation coefficient for CETP B1B1genotype and plasma CETP activity i.e., presence of CETP B1B1 genotype and an increase in the CETP activity would increase the possibility of atherosclerosis. The standardized correlation coefficient for CETP B1B1 genotype i.e., the odds ratio was 2.04 (95% CI 1.011 – 3.150).

DISCUSSION

DISCUSSION

Both genetic and environmental factors play an important role in the pathogenesis of CAD in humans. These factors may vary depending on race and ethnic group. The susceptibility to CAD is a complex trait[171].

Worldwide studies have been done correlating the genetic polymorphisms of CETP with risk for CAD[173,174]. In view of this a comprehensive population based study was performed to determine the association of CETP gene TaqIB polymorphism and its associated CETP activity with Coronary atherosclerosis.

The three human CETP genotypes and the phenotypes were determined in 146 patients with CAD confirmed by angiography and 145 control subjects. The insignificant p value with respect to all the confounding variables like age, sex, BMI, history of diabetes, hypertension, smoking, alcoholism showed that the cases and control groups had been perfectly matched. The significantly low High Density Lipoprotein level (38.5 +9.7), high Low Density Lipoprotein (106 +25.3) in cases re-emphasizes the fact that HDL is protective and LDL causes atherogenesis.

When genotype analysis was performed, distribution of B1B1 genotype was significantly higher among cases (56 (38.4%)) when compared to controls (31 (21.4%)). P value was 0.000 showing that it is significant. This indicates that B1B1 genotype is an independent risk factor for atherosclerosis. There was a significant difference in the distribution of B1B2 genotype also between cases and controls .P value was 0.002.

The evidence available showed that there is a significantly high CETP activity among cases (90.72+15.83) when compared to controls (65.23 + 12.23). P value was 0.000. This shows that high CETP activity is an independent risk factor for atherosclerosis.

When the CETP activity was compared between CETP TaqIB genotypes there was a significantly high CETP activity among B1B1 genotypic individuals (97.09 pmol/μL/hr; p = 0.000 vs B2B2) and B1B2 genotypic individuals (74.53 pmol/μL/hr; p = 0.000 vs B2B2) when compared to B2B2 genotypic individuals (59.10 pmol/μL/hr) . P value was 0.000, suggesting the fact that B1B1 genotype is associated with high CETP activity and this high activity makes a person more susceptible to atherosclerosis. Hence B1B1 genotype and the resultant high CETP activity can be considered as an independent risk factor for atherosclerosis. Due to its intronic location this polymorphism cannot be considered as a part of a functional regulatory site, but can be a marker for another functional site. Its effect on plasma CETP mass, activity and HDL-C can be accounted by its linkage with many 5′ promoter region base changes like −629C>A, −971G/A and −1337C/T polymorphisms. Many large-scale studies have reported an association between CETP activity and the TaqIB polymorphism.

When the High Density Lipoprotein levels were compared between CETP TaqIB genotypes, there was a significantly low HDL level among B1B1 genotypic individuals (37.1 mg/dL; p = 0.000 vs B2B2) and B1B2 genotypic individuals (44.6 mg/dL; p = 0.001 vs B2B2) when compared to B2B2 genotypic individuals (50.2 mg/dL). P value was 0.0002, suggesting the fact that B1B1 genotype is associated with low HDL which makes a person more susceptible to atherosclerosis.

When the Low Density Lipoprotein levels were compared between CETP TaqIB genotypes, there was a significantly high LDL level among B1B1 genotypic individuals (104.9 mg/dL; p value = 0.000 vs B2B2) and B1B2 genotype individuals (91.9 mg/dL; P = 0.05 vs B2B2) when compared to B2B2 genotype individuals (82.3 mg/dL). P value was 0.0004, suggesting the fact that B1B1 genotype is associated with high LDL which makes a person more susceptible to atherosclerosis.

CETP activity correlated negatively with High Density Lipoprotein levels among both cases and controls i.e., decrease in HDL was associated with an increase in CETP activity among both cases and controls.

CETP activity correlated positively with Low Density Lipoprotein levels among both cases and controls i.e., increase in LDL was associated with an increase in CETP activity among both cases and controls.

CETP activity and the number of vessels blocked were found to be strongly positively correlated i.e increase in the activity is associated with an increase in the number of vessels blocked. So CETP activity can also be used to analyze the outcome of atherosclerotic event.

On univariate analysis, the odds ratio for B1B1 genotype was 3.1 (95% CI – 1.8 to 5.4) i.e., a person with B1B1 genotype is 3.1 times at a higher risk when compared to those with B2B2 genotype. The odds ratio for B1B2 genotype was 2.3 (95% CI – 1.3 to 3.8) i.e., a person with B1B2 genotype is 2.3 times at a higher risk when compared to those with B2B2 genotype.

On multiple logistic regression analysis, the negative correlation coefficient for HDL indicates that high HDL level is protective. The positive correlation coefficient for LDL, CETP activity and CETP B1B1 genotype indicates that high LDL, high CETP activity and B1B1 genotype prevalence will increase the susceptibility of atherosclerosis. The odds ratio on multiple logistic regression analysis was 2.04 i.e., a person with B1B1 genotype is 2.04 times at a higher risk for atherosclerosis after all the confounding variables are matched

CONCLUSION

CONCLUSION

- The results of the present study indicate significant association of Cholesteryl Ester Transfer Protein TaqIB polymorphism and the associated high plasma Cholesteryl Ester Transfer Protein activity with Coronary artery disease.

- The Cholesteryl Ester Transfer Protein B1B1 genotype of TaqIB polymorphism and the high plasma Cholesteryl Ester Transfer Protein activity may be independent risk factors for Coronary Atherosclerosis.

SCOPE FOR FURTHER STUDY

SCOPE FOR FURTHER STUDY

- Research aimed at identifying the strategies to inhibit Cholesteryl Ester Transfer Protein activity can be supported.

- Other Cholesteryl Ester Transfer Protein gene polymorphisms may be explored to highlight their association with Cholesteryl Ester Transfer Protein activity and atherosclerosis.

- Various transcriptional factors modulating the Cholesteryl Ester Transfer Protein gene expression can be studied.

- Small interfering RNA (siRNA), based Cholesteryl Ester Transfer Protein gene therapy can be tried in all those who have higher Cholesteryl Ester Transfer Protein activity. But since atherosclerosis is a multifactorial disease, gene therapy does not look promising.

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INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To
Dr. V. Umamaheswari
PG in MD Biochemistry
Madras Medical College, Chennai -3

Dear Dr. V. Umamaheswari

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Association of Cholesteryl Ester Transfer Protein gene Taq1B polymorphism and the Associated Phenotype variation with coronary Artery Disease" No. 05062011.

The following members of Ethics Committee were present in the meeting held on 24.06.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. V. Kanagasabai MD Dean, Madras Medical College, Chennai-3,	-- Deputy chairman
3.	Prof. A. Sundaram, MD Vice Principal , Madras Medical College, Chennai -3	-- Member Secretary
4.	Prof R. Sathianathan MD	-- Member
5.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6.	Prof. Geetha Subramanian MD DM Prof & Head , Dept. of Cardiology, MMC, Ch-3	-- Member
7.	Prof. Pregna B. Dolia MD Director , Institute of Biochemistry, MMC, Ch-3	-- Member
8.	Prof. C. Rajendiran .MD Director , Institute of Internal Medicine, MMC, Ch-3	-- Member
9.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	-- Layperson
10.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
11.	Tmt. Arnold Soulina	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee